EXERCISE AND BONE HEALTH IN WOMEN

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DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or Institute of learning. It has been entirely composed by the author herself.

Jane H. Wilson

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DEDICATION

This thesis is dedicated to my late grandfather, George Law, who was always so enthusiastic about my work.

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The athletes who allowed the author to study them

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CHAPTER 1

ABSTRACT

Part I ***The cross-sectional study***

Objectives To examine the effects of high intensity physical exercise and menstrual irregularity on the bone mineral density (BMD) of both weight-bearing and non weight-bearing skeletal sites in female athletes aged 16 - 65 years and to determine whether intensive weight-bearing exercise can offset bone loss associated with athletic amenorrhoea at appropriate sites.

To assess the influence of morphological characteristics and fitness parameters on BMD in all age groups and to determine whether abnormal eating habits in younger athletes influences BMD.

Subjects The 124 athletes were divided into 3 groups:

Group A 50 middle or long-distance runners aged 16 to 35 years who ran at least 25 miles per week and trained for at least 3 hours per week.

Group B 50 premenopausal veteran athletes aged 40 years and over who were required to have run at least 15 miles per week or trained for at least 3 hours per week for the last three years.

Group C 24 postmenopausal veteran athletes who also were required to have run at least 15 miles per week or trained for at least 3 hours per week for the last three years.

Methods Bone mineral density of lumbar spine (L2-L4) and left proximal femur was measured by Dual Energy X-Ray absorptiometry (DXA) and values were compared between groups and with the European reference range. Morphological measurements included weight, height, body mass index, percentage body fat, fat free

mass. Fitness measurements included maximal oxygen uptake and utilisation (VO_2max), back strength and grip strength. Eating disorders were assessed using the shortened Eating Attitudes Test (EAT26) and the Bulimia Investigatory Test of Edinburgh (BITE).

Results

Group A

Group A comprised top class runners who ran an average of 49 miles per week. The group was subdivided into athletes who were amenorrhoeic (0-3 menstrual cycles per year, AM, n=24), oligomenorrhoeic (4-9 cycles per year, OL, n=9) and eumenorrhoeic (10 - 13 cycles per year, EU, n=17). Bone mineral density was very low in the lumbar spine in the AM group when compared to the EU group (15.6% lower, $p<0.0001$). Surprisingly, BMD of the proximal femur was also significantly lower in the AM group (16.5 - 19.5% lower compared to the EU group, $p<0.0001$). Bone density of the OL group lay between the two other groups.

When compared to the age-matched European reference range, BMD was again significantly lower than expected in the AM group ($p<0.05$ - $p<0.001$). In all three groups, BMD was higher at both sites in the proximal femur than in the lumbar spine, reflecting a positive influence of mechanical stress on BMD in this weight-bearing area. In the athletes who had always menstruated regularly, the benefits of regular running on BMD in weight-bearing bones were demonstrated. In this group BMD was high in the proximal femur but not the lumbar spine compared to the European reference range ($p<0.005$).

Height and weight were consistent independent predictors of BMD in the AM group but not the other groups. Eating disorders were common in the AM group in which

more than 50% were found to have some aspect of disordered eating habits, and poor scoring in the EAT26 was associated with low weight..

Group B was subdivided into athletes who had trained continuously since leaving school (All-timers, AT, n= 23) and those who had started after the age of 30 years (late-starters, LS, n=27). Each group was also divided according to whether they had been continuously eumenorrhoeic (EU) or whether they had had at least two years of prior menstrual irregularity (AM). Most of the athletes were long-distance runners or walkers and the average distance run per week was 32 miles. There were no significant differences between the AT and LS groups in bone density at the sites measured, but both groups showed high bone density compared to the European reference range ($p<0.025$-0.005). Late-starters showed particularly high values in the femoral neck suggesting that even if exercise is commenced after the age of peak bone mass, significant benefits can be achieved.

Those who had remained continuously eumenorrhoeic had particularly high bone density with values 0.4-1.05 SDs above the European age-matched mean. Those who had suffered 1 or more episodes of menstrual irregularity showed smaller benefits in the femur and none in the spine.

Group C was also subdivided as above into all-timers (AT, n=10) and late-starters (LS, n=14). Almost all were long-distance runners and the group ran an average of 30.6 miles per week. There were no significant differences in BMD at any site measured between the AT and LS groups. Nor was BMD in either group different from the European reference range matched for years since menopause (YSM). Bone density (adjusted for YSM) of the proximal femur was associated with weight, body mass index (BMI) and percentage body fat ($p<0.05$) There were no such associations with spinal BMD. Low weight, BMI and percentage fat were associated with high

aerobic capacity (VO_2max). It is postulated that intensive aerobic exercise in the postmenopausal years may be disadvantageous to the skeleton by reducing weight and body fat levels. Less intensive or alternative types of exercise may have more beneficial effects on bone mineralisation

Conclusions Bone density at important fracture sites was substantially greater than expected in women undertaking long-term exercise in the oestrogenized state whether exercise started in the 'teens or the thirties. When endogenous oestrogen levels are low, the apparent beneficial effects of exercise are lost. In postmenopausal women this may be due to low body weight and body fat in those who are the most highly trained. Amenorrhoea in young adult life may have persistent effects on non weight-bearing sites. Individual circumstances must be assessed before exercise can be used as a prescription for the prevention of osteoporosis.

Part 2 The prospective study

Randomised prospective study of the effects of hormone replacement therapy and calcium on bone mineral density in oligo- and amenorrhoeic runners

Objective

To examine the effects of hormone replacement therapy (HRT) and calcium supplementation on bone mineral density in female runners aged 18 - 35 years who had menstrual irregularity.

Method

34 middle and long-distance runners with either oligomenorrhoea or amenorrhoea induced by intensive exercise were randomised to receive A) HRT and 1000mg calcium daily, B) 1000mg calcium daily and C) no treatment, over 18 months. Bone mineral density of lumbar spine and proximal femur was measured by DXA.

Results

Over the first year of treatment there was no difference between the groups in BMD although the trend was for increased BMD in those receiving HRT. When analysed according to whether oestrogen levels had normalised either with resumption of regular mentruation or with HRT, BMD increased in all areas whereas in those who remained amenorrhoeic BMD declined in all areas. The differential change between the 2 groups was significant in all areas except the neck of femur. Only 17 athletes returned for the second follow-up appointment at 18 months and numbers were therefore insufficient for further analysis.

Conclusion

Normalisation of oestrogen levels either by resumption of regular mentruation or by HRT appears to result in an increase in BMD at least over the short term. Larger studies are required to confirm this finding over a long-term period.

CHAPTER 2

INTRODUCTION

Many studies have demonstrated higher bone density in young adults who regularly exercise. The relationship between exercise and bone mineral density (BMD) is marked in athletes (Heinrich *et al.*, 1990, Risser *et al.*, 1990, Wolman *et al.*, 1991, Slemenda *et al.*, 1993) but has also been demonstrated in the general population in those with high levels of normal daily activity (Aloia *et al.*, 1988, Zylstra *et al.*, 1989, Jónsson *et al.*, 1992, Zhang *et al*, 1992). At one time it was expected that the more intense the exercise, the greater the bony response but during the last ten years it has become clear that, in some young women, intensive athleticism is associated with low BMD (Cann *et al.*, 1984; Drinkwater *et al.*, 1984; Marcus *et al.*, 1985, Nelson *et al.*, 1986; Lindberg *et al.*, 1987; Wolman *et al.*, 1990). Such changes in BMD are linked to profound alterations in the hypothalamic-pituitary-ovarian axis resulting in menstrual irregularity such as oligomenorrhoea and amenorrhoea. Although these conditions were initially thought to be relatively benign, it is now known that these hypo-oestrogenic states are associated with reduced bone density. Some regions of the skeleton, for instance the vertebral bodies, may be as much as 25% lower in BMD in amenorrhoeic women compared to their eumenorrhoeic peers. In the short term, musculoskeletal injuries are more common (Lloyd *et al.*, 1986) and concern has arisen that in the long-term there may be a risk of clinical osteoporosis and fracture. Attention has focused on the causes of athletic amenorrhoea in an effort to find ways of reducing its incidence without reducing athletic performance. A greater understanding will also enable sports personnel and physicians to advise more precisely on types and intensity of exercise which will avoid adverse effects on BMD.

The role of exercise in developing peak bone mass, maintaining bone mineral during the premenopausal years and then in the prevention of postmenopausal bone loss appears to be complex. For this and other public health reasons, the promotion of increasing amounts of moderate regular exercise throughout the population now has high priority, but there is uncertainty about how much exercise is moderate and what type of exercise should be proposed. This thesis examines the effect of regular exercise on bone density in four parts:

1) the effect of exercise and menstruation on bone density in young athletes
2) the effect of exercise and menstruation on bone density in women in the decade before the menopause
3) the effect of exercise on bone density in postmenopausal women
4) a pilot study on the treatment of reduced bone density in young amenorrhoeic athletes.

The null hypotheses tested were:

1) Regular exercise has no effect on bone mineral density of the lumbar spine and proximal femur in female athletes of any age
2) Appropriate treatment of runners with mentrual irregularity does not improve bone density.

CHAPTER 3

BACKGROUND

Section 1

Bone metabolism in the general population

Bone mass continues to increase after the closure of the endochondral growth plates and reaches a peak during the second and third decades. Teegarden *et al*, (1995) estimated that by the age of 22.1 +/- 2.5 years, 99% of peak bone mineral density (PBMD) is attained and by the age of 26.2 +/- 3.7 years, 99% of peak bone mineral content (PBMC) is achieved.. In women, factors contributing to the attainment of PBMC include genetic disposition (Hansen *et al.*, 1992, Krall *et al.*, 1993), race (Harris *et al.*, 1995), physical activity (Slemenda *et al.*, 1994, Valimaki *et al.*, 1994), calcium intake (Matkovic *et al*, 1979, Nieves *et al.*, 1995) body mass (Rice *et al.*, 1993, Slemenda *et al.*, 1994, Valimaki *et al.*, 1994, Teegarden *et al*, 1995), alcohol, smoking, medications and underlying disease. Between the ages of 18 and 21, after longitudinal growth ceases, the skeleton goes through a period of consolidation during which it becomes less metabolically active, remodelling driven by osteoclastic resorption reduces and, as a consequence, a moderate amount of additional bone mineral is added to the skeleton by the filling in of remodelling cavities (Parsons et al, 1996).

After the mid-twenties there follows a period of relative stability in bone density until the age of thirty-five to forty years. From then (until the menopause in women) there is normally a slow decline in BMD of some regions of the skeleton, notably the femoral neck (Pearson et al., 1995), but not others (Dequeker et al., 1995). Normally, spinal bone mineral density is stable over this period. .

During the perimenopausal and immediate postmenopausal years there is a phase of accelerated bone loss throughout the skeleton which may initially be as high as 8% per year in trabecular bone (Gotfredson *et al.*, 1986, Reeve et al, 1995). Ravn *et al.* (1994) found that the decrease in BMD of the proximal femur reached values of 9 - 13% in the first 5 years after the menopause. In the spine, bone loss initially averages 3% annually as assessed by dual energy photon or X-ray absorptiometry, but this loss rate is attenuated to only about 1% annually some five years later (Reeve at al, 1995). In Japanese women a decrease in spinal BMD of 20% has been found within 10 years of the menopause. Beyond 10 years the rate of spinal bone loss appears to be very low (Soda *et al.*, 1993, Greenspan *et al.*, 1994). Similarly, in the femur, the rate of loss of bone mineral slows again within 10 years of the menopause to a background 0.5 - 1.0% (Greenspan *et al.*, 1994, Ravn *et al.*, 1994) until old age when it may again start to accelerate in the femur (Jones *et al.*, 1994).

Thus, two types of bone loss seem to occur during the lifetime of a woman. Firstly, from the fourth decade onwards there is a very gradual age-related loss of bone mineral which appears to occur in both cortical and trabecular type of bone. The mechanism by which age-related bone loss is mediated is unknown but it has been likened to the gradual decline in other organs such as muscle mass. Men may suffer from a similar type of bone loss to this first type. In women, at the menopause and for 5 to 10 years afterwards, there is an additional accelerated rate of bone loss which is most marked in trabecular bone. Treatment with oestrogen-containing products at the menopause reduces or eliminates the accelerated loss of bone mineral at this time (Gotfredson *et al.*, 1986, Riis *et al.*, 1987) and it is now accepted that the loss of bone mineral is directly linked to falling sex hormone levels (particularly oestrogen) at the menopause.

It follows that for a given rate of postmenopausal bone loss, the higher the bone mass at the menopause, the less likely that bone density will fall low enough in later years to

result in osteoporotic fracture (Black *et al.*, 1992). An understanding of factors contributing to high bone density is therefore important.. Kröger *et al.*, (1995) estimated that perimenopausal women in the lowest quartile of spinal BMD had a 2.9 times greater risk of fracture than those in the highest quartile. The respective risk for femoral fracture was 2.2 times from the lowest to the highest quartile. Similarly, Cummings *et al.*, (1993) followed more than 8000 women aged 65 years or over for approximately 2 years and found that bone densities of all regions of the hip were closely related to the risk of hip fracture.. Women in the lowest quartile of femoral neck BMD had an 8.5 times greater risk of fracture than those in the highest quartile. For each standard deviation decrease in femoral neck BMD, the increased fracture risk was 2.6 and in the lumbar spine it was 1.6. These findings have been confirmed ina recent meta-analysis of similar studies (Marshall et al., 1996). Also, Cummings *et al.*, suggested that a one SD decrease in femoral neck BMD was associated with an increase in risk of hip fracture equivalent to a 14.5 year increase in age. However it is now clear that rates of bone loss vary rather unpredictably in postmenopausal women so that a measurement of BMD made at menopause may not accurately predict risk 10 or 20 years later (Hui et al 1990).

Bone mineral density of the spine and femur has been shown to be correlated with muscle strength (Pocock et al., 1989), most reports of such relationships concerning older non-athletic women. Sinaki et al. (1989) found a significant positive relationship between BMD of the lumbar spine and back extensor strength in postmenopausal women which held true even after adjustment for age. In a large study of 709 and 1080 elderly men and women respectively, quadriceps strength was found to predict bone density in the proximal femur in men only (Nguyen *et al.*, 1994). However when age and weight-adjusted BMD of the femoral neck was analysed, in tertiles of muscle strength and calcium intake, those in the highest tertiles had approximately 5% greater bone density than those in the lowest tertiles. This was true for both men and women.

In younger women, femoral neck and spinal BMD have been found to correlate with both quadriceps and hamstring and trunk extensor strength (Eickoff *et al.* 1993). Grip strength has been found to correlate well not only with forearm BMD but also with BMD at distal sites in elderly women (Bevier *et al.*, 1989, Kritz-Silverstein & Barrett-Connor, 1994). It is likely that in this group of women at least, grip strength reflects habitual level of daily activity. It should be remembered that such associations do not necessarily imply causality.

Much interest has naturally centred on factors governing the attainment of maximal potential peak bone density, maintenance of bone density in the premenopausal years and on the minimisation of the post-menopausal loss of bone mineral. Encouraging higher levels of physical activity within the general population might go some way towards reducing the fracture epidemic, particularly in the elderly (Cooper, 1995) but advice must be specific and capable of being complied with for it to be readily practical. In the future, long-term prospective randomised studies on exercise will hopefully provide the required information. In the meantime, studies on athletes show what can be achieved with relatively high levels of exercise.

Section 2

The effect of exercise and mechanical forces on bone

Julius Wolff (1892) first suggested that bone responds to mechanical stress to increase strength at areas of high strain. Ground reaction forces during weight-bearing activity are therefore likely to provide a suitable stimulus for bone remodelling. Indeed, it is well recognised that immobility due for example to bedrest, spinal cord injury or weightlessness (in space) are associated with rapid and sometimes, profound losses in bone mineral.

However, the mechanical strain concept of bone remodelling suggests that mechanical stress applied to bone will stimulate increased bone mass at that site even in the absence of ground reaction forces. This is supported by studies on non weight-bearing bones. National level Finnish squash players had 15.6% greater BMD in the humerus of the playing side compared to the non-playing side (Haapasalo *et al.*, 1994) and in tennis players, BMD is higher in the dominant arm than the non-dominant side (Dalen *et al.*, 1985; Pirnay *et al.*, 1987). In a study of National level female rowers and non-rowing athletes, back strength was greater in both flexion and extension in the rowers and bone density of the lumbar spine was correspondingly higher in this group even when adjusted for body weight (Wolman *et al.*, 1990).

The number and weight of loading cycles applied to bone are probably as important as the type of exercise undertaken. Rubin and Lanyon (1984) and Lanyon (1996) have shown that, in experiments with some animal models, a maximal adaptive response can be engendered by a very small number of cycles of increased strain with no additional benefit gained by increasing the number of loading cycles providing the peak strain achieved was sufficiently high. Frost's 'Mechanostat' theory of bone remodelling requires that a minimum effective strain must be exceeded in order to initiate the modelling process and thus promote an increase in bone mass driven by increased bone formation (Frost, 1992). This suggests that the mechanical adaptability of bone is best suited to respond to dynamic events of short duration and high intensity rather than low intensity activities repeated over a long period of time. If this applies in humans one might expect higher bone density in those performing short duration, high load exercise than in those involved in predominantly aerobic, low intensity exercise.

Until recently the means by which mechanical strain is perceived by bone and converted into messages controlling bone remodelling has been little understood. Recent work has shown that one type of bone cell, the osteocyte, can perceive the application of mechanical forces. Osteocytes are found in lacunae in bone and communicate with one

another and with other cells in bone by means of dendritic processes which traverse the canaliculi of bone. It is thought that nutrients and signalling molecules are carried by 'streaming' of the extracellular fluid and changes are detected by the osteocyte. This mechanism is enhanced by minute deformations of the bone matrix of the order of 0.2% (cortical bone) or 0.5% (trabecular or cancellous bone) (Lozupone 1996).

The mechanism by which osteocytes promote bone formation and resorption at selected locations is less clear. El Haj et al., (1990) have demonstrated that after mechanical stimulation there is a rapid, transient increase in the enzyme glucose 6-phosphate dehydrogenase followed by a more prolonged production of prostaglandins which can promote both bone formation and bone resorption in different circumstances. Bone resorption is inhibited by repeated deformations of as little as 200-400 μstrain (0.02-0.04%) (Lanyon 1990), whereas strains capable of promoting bone formation need to be 10 fold larger. This level of activity in humans in most likely to be acheived by children or athletes, particularly those performing strength exercises.

Until recently it was felt that the activity in cells involved in bone resorption (osteoclasts) was closely 'coupled' with the cells which form new bone (osteoblasts). In this way new bone formation followed bone resorption, with osteoclasts and osteoblasts working together in teams (Eriksen 1986). However it is now known that in some situations, cells lining the mineral matrix of bone can transform back into osteoblasts without associated activity in osteoclasts. It is possible that this occurs after mechanical stimulation and is the mechanism by which more rapid alteration of bone matrix can be achieved.

a) Young athletes

i) Cross-sectional studies

BMD of the femur, pelvis, tibia and os calcis have all been shown to be high in athletes undertaking weight-bearing sport (Heinrich *et al.*, 1990, Risser *et al.*, 1990, Wolman *et al.*, 1991, Slemenda *et al.*, 1993) when compared to sedentary controls. Of all sports, weight-lifting is likely to provide the greatest stimulus to increase bone formation in the spine. Granhed *et al.*, (1987) calculated that the load applied to the third lumbar vertebra during a single dead lift during the mens' World Championships in Power Lifting was as high as 36.4 kN. Bone mineral content of the lumbar spine was extremely high in the lifters compared to age and weight-matched sedentary controls (7.06, SD 0.87 $g.cm^{-1}$ vs. 5.18, SD 0.88 $g.cm^{-1}$). There was also a close correlation between the estimated annual weight lifted and BMC. Similar results were found by Conroy *et al.*, (1993) and Virvidakis *et al.* (1990), in elite junior weight-lifters. Studies comparing women who lift weights with those who run, swim or cycle have found that BMD is significantly higher in the lifters compared to sedentary control groups and the other exercisers (Davee *et al.*, 1990, Heinrich *et al.*, 1990, Heinonen *et al.*, 1993).

Bone mineral density of the spine and femur has been shown to be correlated with muscle strength (Pocock et al., 1989) though most reports of such relationships concern older non-athletic women. Sinaki and Offord (1988) found a significant positive relationship between BMD of the lumbar spine and back extensor strength in postmenopausal women which held true even after adjustment for age. In a large study of 709 and 1080 elderly men and women respectively, quadriceps strength was found to predict bone density in the proximal femur in men only (Nguyen *et al.*, 1994). However when age and weight-adjusted BMD of the femoral neck was analysed, in tertiles of muscle strength and calcium intake, those in the highest tertiles had approximately 5% greater bone density than those in the lowest tertiles. This was true for both men and women. In younger women, femoral neck and spinal BMD have also been found to correlate with both quadriceps and hamstring and trunk extensor strength (Eickoff *et al.*

1993). It should be remembered that such associations do not necessarily imply causality.

Grip strength has been found to correlate well not only with forearm BMD but also with BMD at distal sites in elderly women (Bevier *et al.*, 1989, Kritz-Silverstein & Barrett-Connor, 1994). It is likely that in this group of women at least, grip strength reflects habitual level of daily activity. Because of its relative ease of measurement, grip strength might in future prove suitable as a contributing measurement in an overall screening programme to detect those at risk of osteoporosis.

ii) Exercise programmes

Cross-sectional studies on athletes and the general population have demonstrated relationships between bone density and type and intensity of exercise but it is possible that these correlations are the result of selection bias, reflecting a likelihood that those with higher bone density will exercise more intensively. Only prospective randomised studies of exercise programmes can eliminate this possibility. Extremely intensive weight-bearing training in military recruits has been found to result in marked increases in bone density in the tibia (5.2 - 11.1%) over as little time as fourteen weeks. However, the drop-out rate due to stress fractures was high at 40% and this type of training is unsuitable for most of the population (Margulies *et al.*, 1986). A more suitable supervised aerobic exercise and weight-training programme of 35 weeks duration in previously untrained college women was shown to result in small but significant increases in lumbar BMD with no detectable changes in the femur (Snow-Harter *et al.*, 1992). In a similar study performed over 2 years, small gains in BMD were found in both spine and femur (Friedlander *et al.*, 1995). Even in previously trained gymnasts, an increase in lumbar BMD has been noted with short term increased training programmes (Nichols D.L., 1994). Other short-term studies support the relationship between exercise programmes and beneficial effects on bone density in young adults (Bassey &

Ramsdale, 1995) but the effect may be short-lived with return to baseline of bone density after only 3 months of detraining (Vuori *et al.*, 1994).

b) Bone density and exercise in children and adolescents

The effects of exercise on BMD during the prepubertal and pubertal years may be less marked than in later adolescence. Slemenda and Johnson (1993) compared 44 young female skaters and non-skaters aged 10 to 23 years. Bone density was higher in the skaters but this difference was not apparent until about the age of 15 years. Alternatively, this might have reflected overall levels of commitment to the sport and length of participation at different ages. Kroger *et al.*, (1993) found no relationship between bone density or annual rate of increase of spinal and femoral BMD and levels of sporting activity in 65 Finnish children aged 7 - 20 years. In contrast, Slemenda et al (1994) found a more substantial effect of physical activity in boy twins participating in a study of calcium supplementation than they did for calcium. Grimston *et al.*, (1992) were able to demonstrate higher BMD in 13 year old boys who trained intensively for weight-bearing sports compared to swimmers but this trend was less evident in the girls. Similarly, in a 15 year longitudinal study of 98 females and 84 males aged 13 - 27 years, BMD of the lumbar spine at age 27 years was correlated with amount of weight-bearing activity during the preceding years in males but not in females (Welten *et al.*, 1994). Cooper et al (1995) found that BMD in a population-based study of young women was correlated with past and present levels of physical activity.

Section 3

Menstrual irregularity and bone density in athletes

Normal bone metabolism requires an intact hypothalamic-pituitary-ovarian axis. In women the key hormones are oestrogen and progesterone. Oestrogen prevents bone resorption and decreases remodelling whereas progesterone promotes bone formation and accelerates remodelling. In the normal hormonal milieu, bone resorption and

formation are 'coupled' so that appropriate remodelling can occur as required. In the presence of low levels of oestrogen and progesterone, resorption increases since bone formation is insufficiently increased to compensate, with a net loss of bone mineral. Even asymptomatic disturbances of ovulation such as anovulation or a short luteal phase have been associated with bone mineral losses of up to 4% per year (Prior *et al.*, 1990). Many female athletes have disruption of their menstrual cycle and this has now been recognised to have profound effects on bone mineral content.

a) Incidence of menstrual abnormalities

Short luteal phases, anovulatory cycles, oligomenorrhoea and amenorrhoea have all been described in athletes. Whereas secondary amenorrhoea occurs in only 5% of the general population it is common in sports in which thinness may be an added advantage to performance, such as running, gymnastics, ice-skating and dancing. In the Great Britain National squads of these sports in 1988, 50-100% of women reported at some time previously some form of menstrual dysfunction (Wolman & Harries, 1989). The incidence may also be high in other sports. Recently, Güler and Hasçelik (1993) demonstrated an overall rate of 30% for oligomenorrhoea and amenorrhoea amongst top athletes in team games from a number of different countries. Volleyball players from Czechoslovakia, Belgium and Sweden had the highest rates at 43%. The term athletic amenorrhoea has now come into use to describe secondary amenorrhoea associated with intense exercise .

b) Causes of menstrual disturbance

i) Severity of training

There is no doubt that the aetiology of athletic amenorrhoea is multifactorial. Increasing severity of training has been shown in some studies to correlate with increasing incidence of menstrual dysfunction (Feicht *et al.*, 1978, Dale *et al.*, 1979, Drinkwater *et al.*, 1984, Drinkwater *et al.*, 1986, Güler and Hasçelik, 1993). Others have been unable

to confirm this relationship (Glass *et al.*, 1987) and it is clear that there are other factors correlated with intensity of training which must be accounted for.

ii) Body Composition

Amenorrhoeic runners have been found to be lighter and leaner than their eumenorrhoeic peers (Feicht *et al.*, 1978, Schwartz *et al.*, 1981, Shangold & Levine, 1982, Marcus *et al.*, 1985; Drinkwater *et al.*, 1986, Glass *et al*, 1987, Harber *et al.*, 1991). It is well known that profound weight loss, as occurs in anorexia nervosa, is associated with amenorrhoea and it seems likely that low weight or changes in body composition in some way contribute to the development of this disorder in athletes. But as yet the exact relationship remains obscure.

iii) Menstrual history

There may be a relationship between age at start of training, age at menarche and likelihood of later menstrual irregularity. Güler & Hasçelik (1993) found that of the 96 top team-game athletes sampled, those with menstrual dysfunction tended to be younger, had started training at an earlier age and were more likely to have started training before menarche. In elite speed skaters, those who reported prolonged intermenstrual intervals also had the highest average age at menarche (Casey *et al.*, 1991). It has also been shown that women with menstrual irregularity prior to the start of training are more likely to develop irregularity during training (Schwartz *et al.*, 1981, Shangold & Levine, 1982). This suggests that immaturity of the hypothalamic-pituitary-ovarian axis may make the development of menstrual irregularity more likely at times of psychological or physiological stress.

iv) Nutritional intake

Several recent reports have suggested that the calorie intake of amenorrhoeic athletes may be lower than that of eumenorrhoeic subjects and that restriction of energy intake may be causally related to menstrual dysfunction. Nelson *et al.* (1986) used a three-day dietary record to compare the intakes of 11 amenorrhoeic and 17 eumenorrhoeic highly trained runners. They reported the mean energy intake of amenorrhoeic runners to be

520 kcal.day^{-1} less than eumenorrhoeics. The difference in calorie intake was highly significant ($P<0.005$) when expressed as a function of lean body mass. Baer (1993) also found lower intakes ($P<0.05$) in amenorrhoeic runners compared to both eumenorrhoiec runners and controls (1627 +/- 75, 1944 +/- 45, 1950 +/- 56 kcal.day^{-1} respectively). Five out of a further seven studies on runners have shown lower total calorie intakes in the amenorrhoeic athletes but these differences have not been statistically significant (Brownell *et al.*, 1988, Rosen & Hough, 1988, Kaiserauer *et al.*, 1989, Rucinski, 1989, Prussin & Harvey, 1991). In only two studies has calorie intake been shown to be higher in amenorrhoeic athletes compared to eumenorrhoeic athletes (Snead *et al.*, 1992; Wilmore *et al.*, 1992).

v) Eating disorders

The current literature suggests a high incidence of anorexic or bulimic types of behaviour in some athletes, particularly those competing in events in which low weight conveys an advantage or which are judged on aesthetic appeal (Weight & Noakes, 1987, Brownell *et al.*, 1988, Rosen & Hough, 1988, Rucinski, 1989, Mulligan & Butterfield, 1990, Prussin & Harvey, 1991). Preoccupation with weight and food, a desire to maintain a low body weight or body fat and intensive exercise are all hallmarks of anorectic behaviour. Both anorexia and bulimia are associated with menstrual disturbance and it may be that disordered eating rather than energy intake per se is the main contributor to the high incidence of amenorrhoea.

Section 4

The effects of menstrual irregularity on bone

a) Menstruation and bone density of non weight-bearing bones

During the last decade several studies have examined the effect of menstrual irregularity on BMD. Studies on amenorrhoeic runners compared to either eumenorrhoeic runners or sedentary controls have been consistent in showing lumbar BMD to be 10-20% lower in those with amenorrhoea (Cann *et al.*, 1984; Drinkwater *et al.*, 1984; Marcus *et*

al., 1985, Nelson *et al.*, 1986; Lindberg *et al.*, 1987; Wolman *et al.*, 1990). In the largest study of its kind, Drinkwater *et al.*, (1990) demonstrated a linear relationship between vertebral BMD and lifetime menstrual history in 97 active women. Those who had a long history of oligo/amenorrhoea had a mean BMD which was 17% lower than in those who had always had regular periods. A linear relationship between lumbar spine BMD and oestradiol has also been demonstrated by Nelson *et al.*, (1986) in amenorrhoeic and eumenorrhoeic runners.

The effect of hypo-oestrogenism may not be as marked in other athletes as in runners. Young *et al.*, (1994) found a significant but small reduction of only 3.5% in the lumbar spine of ballet dancers with slightly greater reductions of 5 - 6% in ribs, arms and skull when compared to non-dancers with regular menstrual cycles. Robinson *et al.*, (1995) showed that runners had 12% lower lumbar BMD than controls, whereas values in gymnasts were 5.5% higher than controls, despite a similar prevalence of oligoamenorrhoea in the two athletic groups. The gymnasts were much stronger generally than the runners or controls and it is possible that the greater muscular stresses applied to the spine in gymnasts help to offset any reduction in bone mineral due to menstrual dysfunction. Certainly in rowers this seems to be the case. Wolman *et al.*, (1990) found that in a group of elite athletes, rowers with prolonged amenorrhoea had higher lumbar bone density than non-rowers and rowers with or without menstrual dysfunction had significantly greater back strength than amenorrhoeic runners.

b) Menstruation and bone density of weight-bearing bones

There is less agreement about the effect of amenorrhoea on weight-bearing bones in which the muscular stresses of exercise and the effect of ground reaction forces may offset bone loss. Reduction in femoral shaft BMD in amenorrhoeic active women has been noted by one author (Drinkwater *et al.*, 1990) but Wolman *et al.* (1991) found no difference in femoral shaft BMD between amenorrhoeic and eumenorrhoeic runners,

rowers and dancers. Femoral neck, calcaneal and total leg BMD have also been shown to be well maintained despite amenorrhoea (Drinkwater *et al.*, 1990; Harber *et al.*, 1991, Myerson *et al.*, 1992; Snead *et al.*, 1992, Young *et al.*, 1994). However, in Robinson's study (1995) lower femoral neck BMD was found in a group of 20 runners (of whom 30% were oligo- or amenorrhoeic) compared to eumenorrhoeic sedentary controls. When analysed according to more detailed menstrual history there was a 17% difference between those runners who had always had regular menstrual cycles and those who had had prolonged amenorrhoea. Numbers in each group were too small for more detailed analysis. More recently, studies from Drinkwater's group have also found lower BMD in all femoral areas measured, thus refuting findings from the earlier study (Rencken et al., (1996). This suggests that there may be a linear relationship between number of yearly menstrual cycles and bone density of other skeletal regions in addition to the spine.

More recently controversy has arisen about whether bone density should be expressed according to body weight. Most population studies have shown that body mass is a significant independent predictor of bone density: those who are lightest have the lowest bone density. This is of particular relevance in athletes who are in general lighter than their sedentary peers. Amenorrhoeic athletes tend to be even lighter still. Some authors have therefore analysed their data after adjustment for body mass. Myerson *et al.*, (1992), Robinson *et al.*, (1995) and Young *et al.*, (1994) all found that previously noted differences between amenorrhoeic and eumenorrhoeic athletes were eliminated when this method was used. Only Drinkwater *et al.*, (1990) found a continuing trend towards reduced lumbar spine BMD even when adjusted for weight. It is possible that in athletes, weight only becomes a significant predictor of bone density in the presence of very low levels of sex hormones.

Section 5

Mechanism of reduced bone density in athletic amenorrhoea

Amenorrhoea in athletes is a form of hypothalamic hypogonadism. Pulsatile releases of luteinising hormone (LH) and follicle stimulating hormone (FSH) are reduced in amenorrhoeic athletes (Loucks *et al.*, 1989). Some normally menstruating runners also have reduced LH pulsatility (Cumming *et al.*, 1985, Loucks *et al.*, 1989). Gonadotrophin releasing hormone (GnRH) stimulation studies have revealed an exaggerated response of the pituitary to exogenous GnRH in amenorrhoeic athletes (Vedhuis *et al.*, 1985, Loucks *et al.*, 1989). This implies that GnRH production by the hypothalamus is suppressed whilst pituitary function is retained. Reduced LH and FSH pulsatility means that the ovaries do not prepare a follicle for release, ovulation does not occur and there is no corpus luteum. Levels of oestrogen and progesterone may remain at early follicular levels throughout the cycle even if normal menses are present.

Very few investigators have been able to study the changes in bone occurring at the microscopic level in such athletes. However, Warren *et al.*, (1990) performed a bone biopsy on a 20 year old dancer with long-standing anorexia and primary amenorrhoea who suffered a femoral head collapse. She was known to have lumbar BMD which was more than 2 standard deviations below the normal mean for her age. On microscopy, femoral cortical bone was markedly reduced in thickness and increased in porosity. At the trabecular level, the resorption surfaces were greatly increased although the formation surface was normal. It is unknown whether such changes occur in all amenorrhoeic athletes, the rate at which they might occur or whether they are reversible with resumption of menses or treatment with appropriate hormones.

Section 6

Consequences of low bone density in athletes

a) Stress fractures

Lloyd *et al.* (1986) reviewed the medical records of 207 collegiate athletes and found X-ray confirmed fractures (type of fracture not defined) in 9% of regularly menstruating women and 24% of women with irregular or absent menses. In dancers, two papers report a relationship between bone injuries or stress fractures and amenorrhoea (Warren *et al.*, 1986; Benson *et al.*, 1989) and a survey of 240 female athletes showed a higher incidence of stress fractures in those with fewer than 5 menses per year (49%) compared to those with 10 or more menses per year (29%) (Barrow & Saha, 1988). Lindberg *et al.*, (1984) found that 49% of the amenorrhoeic runners in his study had had stress fractures in the previous year whereas none of the eumenorrhoeic runners had suffered such injuries. However, two reports found no significant relationship between menstrual history and stress fractures although both contained small numbers of subjects and so lacked statistical power (Frusztajer *et al.*, 1990; Grimston *et al.*, 1990). Recognition of the possibly increased risk of stress fractures in amenorrhoeic women is important because delay in diagnosis can result in full thickness fracture (Leinberry *et al.*, 1992).

It remains unclear whether the increased rate of stress fractures in amenorrhoeic athletes is related to low BMD. Femoral bone density has been shown to be low in young male military recruits with femoral stress fractures (Pouilles *et al.*, 1989) and Myburgh *et al.*, (1990) found that in athletes with similar training habits, those with stress fractures were more likely to have lower femoral neck and spinal bone density, lower dietary calcium intake, current menstrual irregularity and lower oral contraceptive use. But Carbon *et al.* (1990) assessed elite female runners with and without stress fractures and found no difference in the femoral BMD between the two groups. Others have also described a lack of association between tibial BMD and

stress fractures in military recruits (Milgrom *et al.*, 1989). It is likely that the association between amenorrhoea and stress fractures is due to impaired microfracture healing, in the absence of adequate levels of oestrogen, rather than decreased mechanical integrity. In hypooestrogenic states such as after the menopause or during amenorrhoea, there is an increase in programmed cell death (apopoptosis) of osteocytes (Tomkinson 1996). This change may affect the ability of bone to transduce mechanical forces and to initiate the repair of microscopic cracks which occur even under normal circumstances in spinal trabeculae and the femoral head and neck from at least middle age onwards. If oestrogen suppression during athletic amenorrhoea leads to osteocyte apoptosis, there may be a consequent disruption of the signalling pathways responsible for micro-fracture repair, leading to crack enlargement and the development of a clinical stress fracture.

b) Musculoskeletal injuries

Stress fractures may not be the only injury more prevalent in amenorrhoeic athletes. Participants in a 10km race who responded to a questionnaire were more likely to have taken time off training due to any form of musculoskeletal injury if they had irregular menses (Lloyd *et al.*, 1986). In Benson's study (1989) of 49 female dancers, those with abnormal menses had more 'bone injuries' (mean = 15.0) than normally menstruating dancers (mean = 5.0) ($P<0.05$). Additionally, dancers with a low body mass index (<19.0 kg.m^{-2}) had a greater duration of low-grade musculoskeletal injury (mean = 24.1 days) than those with a higher BMI (mean = 11.6 days) ($P<0.05$).

More severe bony injury also occurs in amenorrhoeic athletes. In dancers, scoliosis was found to be more common in those with delayed menarche and in whom anorectic behaviour was more prevalent (Warren *et al.*,1986). Warren *et al.* (1990) later described a 20 year-old ballet dancer with long-standing anorexia nervosa, primary amenorrhoea and low BMD, who suffered femoral head collapse. Recently we have

reported an osteoporotic fracture in the neck of humerus of a 30 year old marathon runner with a history of anorexia and low bone density (Wilson & Wolman, 1994).

c) Long-term consequences

Amenorrhoeic athletes may be at risk of premature osteoporosis and fractures but as yet there is little long-term data on the natural history of bone metabolism in this condition. As previously shown, bone mass peaks at approximately 20 - 30 years of age so athletes should be at their highest BMD during their most athletic years. Eumenorrhoeic athletes have higher peak BMD (PBMD) than the mean for the population. But if amenorrhoeic athletes fail to attain their maximum potential PBMD at this age, it is unknown whether they will be able to 'catch up' later in life. If the lifetime risk of hip fracture is related to peak bone mass, eumenorrhoeic athletes are likely to be at reduced risk of osteoporotic fracture throughout their lifetime whereas amenorrhoeic athletes may always be at a relative disadvantage.

But BMD may increase when menstruation returns in these athletes. Drinkwater *et al.* (1986) followed 9 athletes over a 15.5 month period. Seven of the women had regained menses and two had remained amenorrhoeic. Lumbar BMD increased by 6.3% in the former amenorrhoeic women whilst decreasing a further 0.3% in those who had remained amenorrhoeic. Small increases in BMD were also seen in the radius. These results are very similar to those of Lindberg *et al.* (1987) who retested seven amenorrhoeic runners at 15 months. Four had recovered menses and showed an improvement of 6.5% in lumbar BMD. The other three remained amenorrhoeic and showed no improvement in BMD.

Some data are available from anorexics with amenorrhoea. Rigotti *et al.*, (1991) followed cortical bone density of the radius in non-athletic patients with anorexia nervosa over a median of 25 months. Only 6 of the 27 women regained menses

although most gained some weight, took calcium supplements and exercised regularly. There were no significant changes in BMD in women who regained menses, received oestrogen therapy or who gained weight to more than 80% of their ideal weight. In addition there was a high incidence of vertebral compression fractures and non-spine fractures. Weight gain however and either oestrogen therapy or return of menses in anorexics has been shown to be associated with improvements in spinal BMD by Bachrach *et al.*, (1991). Studies on recovered anorexics also suggest that total body BMD may return towards normal for the age (Treasure *et al.*, 1987, Bachrach *et al.*, 1991).

These short-term studies suggest that small improvements in BMD may occur in some regions of the skeleton, particularly those high in trabecular bone such as the spine, when menstruation returns. However, changes in cortical bone may not occur so rapidly, if at all. It is also unknown whether improvements in trabecular bone are maintained over several years.

There is some evidence from cross-sectional studies of older premenopausal and postmenopausal women that gynaecological parameters may have important associations with BMD. Greater numbers of pregnancies, early menarche and greater numbers of days bleeding have all been correlated with higher radial BMD (Fox *et al.*, 1993). In this study, for each decade of menstruation, radial BMD was 2% higher but there was no association with length of the menstrual cycle or previous history of irregularity. This contrasts with findings by Georgiou *et al.*, (1989) who found that BMC of the forearm in postmenopausal women had a better linear correlation with the total number of menstrual cycles than with age or years since the menopause. Only long-term prospective studies of amenorrhoeic athletes will be able to confirm or refute the relationship between these various aspects of menstrual history and their influence on BMD in the postmenopausal years.

Section 7

Treatment of reduced bone density in athletes

Drinkwater *et al.* (1986) and Lindberg *et al.* (1987) demonstrated an increase in BMD associated with resumption of menses but in all cases return of menses was associated with a reduction in training volume or intensity and a concomitant increase in weight. Not all athletes are willing to alter training habits in order to resume menstruation. For many, menstruation would be a nuisance and for some it might interfere with performance. In such athletes treatment to prevent further bone mineral loss or to improve low bone density despite amenorrhoea would be of interest. Treatment regimes used in women with secondary amenorrhoea not willing to resume monthly menstruation have included calcium supplementation, recently introduced forms of hormone replacement therapy such as continuous combined regimes, and intranasal calcitonin.

a) Hormone replacement therapy

De Cree *et al.*, (1988) used 2mg cyproterone acetate and 50μg ethinyl oestradiol as a combined oral contraceptive in 7 amenorrhoeic athletes. During 8 months of treatment, BMD in the lumbar spine increased by 9.5% compared to a control group of 4 athletes in whom BMD increased by only 1.6%. Very little change occurred in either group in the radius. It is difficult to assess the effect of the cyproterone acetate on bone density, indeed the increase may have been solely due to the relatively high dose of oestrogen used. In a study of 15 non-athletic women with primary and secondary amenorrhoea, Haenggi *et al.*, (1994) showed increases of 2.5 % and 2.9% per year in lumbar spine and Ward's triangle respectively when treated with an oral contraceptive containing 0.03mg ethinyloestradiol and 0.15mg desorgestrel. Non-significant increases in BMD were observed in the femoral neck and tibia suggesting that greatest effects occur at sites high in trabecular bone. Similarly treatment with medroxyprogesterone in physically active women with menstrual cycle disturbance has shown significant

improvements in spinal BMD (Prior *et al.*, 1994). But treatment with oestrogen and progestins in anorexia nervosa patients with proven low spinal trabecular BMD have given conflicting results. Only those with body weight less than 70% of ideal appeared to derive benefit from treatment in that further bone loss was prevented compared to controls. The greatest changes were seen in those controls who regained menses in whom there was a 19.4% increase in BMD (Klibanski *et al.*, 1995).

b) Calcium

Treatment with calcium has been suggested to be weakly effective by Prior *et al.*, (1994) in his study of 61 active women but no effect has been shown by others. Baer *et al.* (1992) treated 7 adolescent amenorrhoeic runners with 1200 mg calcium carbonate and 400 IU vitamin D daily. During a 12 month period the subjects consumed an average of 2400 mg calcium daily but BMD of the lumbar spine did not increase and in two subjects it declined further.

c) Intranasal calcitonin

In non-athletes with menstrual abnormalities, intranasal calcitonin and oestrogen or progesterone replacement have been used. In 7 women with primary amenorrhoea who completed 6 months of treatment with either intranasal calcitonin or a combined oestrogen/progesterone therapy, BMD of the lumbar spine increased by 4.1% in the first group and by 9.2% in the latter group (Biberoglu *et al.*, 1990).

d) Bisphosphonates

As yet there are no studies on the effect of the bisphosphonates on low bone density in amenorrhoeic athletes. They are now becoming widely accepted as a treatment of choice for established osteoporosis in postmenopausal women (Black 1996) but their role in hypogonadal bone loss in premenopausal women is conjectural.

Although some mention was made in these studies on the incidence of side effects with treatment there was no mention made of effects of treatment on athletic performance. Side effects associated with hormone therapy by women who have experienced prolonged amenorrhoea such as breast tenderness, weight gain and emotional lability are unlikely to be tolerated by runners. Calcium in high doses may also cause troublesome gastrointestinal upsets in a minority, which is particularly difficult for runners.

Effective treatment of reduced bone density in young women seems possible, at least in the short-term, though side effects may be a particular problem in athletes and may reduce compliance. Long-term effects of treatment are unknown and must await further investigation. A more effective approach to the problem must be in the prevention of athletic amenorrhoea with prompt assessment and treatment should it occur.

Section 8

Bone density in perimenopausal and postmenopausal women

A dramatic consequence of the ageing of the population has been the enormous increase in the numbers of osteoporotic fractures. Although undoubtedly vertebral and radial fractures cause considerable morbidity, it is the mortality and cost associated with hip fractures that cause particular concern to the health planners. The mortality rate at one year following fracture is almost 20% and in those who survive, very few regain their previous level of mobility. Based on past trends in hip fracture rate it is estimated that the total number of cases (men and women) will rise from about 50,000 in 1985 to almost 120,000 in 2016 (Kanis & Pitt, 1992).

Vertebral fractures are harder to quantify as many are asymptomatic but Melton *et al.*, (1989) suggested that the prevalence may be as high as 26.5% in all women aged over 55 years. A report from the USA based on 1986 data estimates that vertebral fractures

accounted for 10% of all osteoporosis-related hospital admissions in women over the age of 45 years (Phillips *et al.*, 1988). In Europe, rates of prevalent deformities in population samples of men and women aged over 50 (mean age approximately 65 years) were in excess of 10%. However, not all vertebral deformities are due to osteoporosis (O'Neill et al, 1996).

a) The role of exercise in preventing osteoporosis and fracture

Prevention of osteoporosis and its devastating consequences has become a key issue and much effort has been devoted to an understanding of its aetiology. The incidence of hip fractures is directly related to bone strength, the risk of falling and the neuromuscular responses to falling. The higher the bone density in early adult life, the lower the risk of fracture in later years. Those women with greater bone density at the menopause are also at lower risk of fracture. Cooper *et al.*, (1988) showed that increased daily activity protected against fracture in men and women aged 50 years and over. Muscle power was independently associated with risk of fracture with a five-fold increase between the lowest and highest fifths of strength, and was significantly related to all measured indices of activity. In a 15 year prospective study of 1419 elderly men and women in Britain, Wickham *et al.*, (1989) showed that the relative risk of hip fracture was 5.2 in those who were housebound compared to those with full outdoor activity. Physical activity such as household work, strenuous conditions of employment and sport during early adult years also appears to protect women from later fracture, regardless of level of activity at the time of fracture (Åstrom *et al.*, 1987).

A greater understanding of mechanisms by which bone mass in old age can be improved, and the risks of falling reduced, will help to focus preventative programmes. Regular exercise may be one method of maintaining BMD, preventing the development of osteoporosis and reducing the rate of falling. However, the amount of exercise

required to have a maximal effect is unknown. Nor is it known whether exercise in the elderly is as beneficial as activity in the early adult years.

b) Cross-sectional studies

Population-based studies in older women have consistently shown higher bone density in those women who are moderately active compared to those who are sedentary (Aloia *et al.*, 1988, Zylstra *et al.*, 1989, Jónsson *et al.*, 1992, Zhang *et al*, 1992). For instance, Krall & Dawson-Hughes (1994) found higher whole body BMD in postmenopausal women walking approximately 1 mile per day compared to those walking shorter distances. The rate of bone loss in these active postmenopausal women was also slowed.

Two cross-sectional studies on older 'athletic' women have also shown higher BMD than age-matched non-exercisers. In both studies women were considered athletic if they exercised for at least 1 hour, three times a week at the equivalent intensity of a game of tennis. The most significant differences in bone density occurred in the oldest, postmenopausal women in whom bone density was maintained compared to the control groups in whom it declined (Jacobson *et al.*, 1984, Talmage *et al.*, 1986).

Thus regular activity in older women appears to be associated with significant skeletal benefits. However, very intensive exercise in postmenopausal women may not have the same advantages. Michel *et al.*, (1989) found that women exercising for more than 5 hours per week had quite low lumbar BMD compared to other women who trained less intensively. In a 2 year prospective follow-up study to this, Michel *et al.*, (1991) confirmed that, in females over the age of fifty, extreme levels of exercise (greater than 300 minutes per week) were associated with low bone density. And Nelson *et al.*, (1988), found that endurance-trained postmenopausal women (running 22.6 +/- 2.4 miles per week) had surprisingly similar lumbar, femoral and radial BMD to age-

matched sedentary women, although, when adjusted for weight, lumbar and radial bone density was higher in the active group.

c) Exercise programmes

The evidence for beneficial effects of exercise intervention programmes on bone density in postmenopausal women is conflicting. Most studies have involved small numbers of subjects for a maximum of one year. In some, the type of exercise has been inappropriate to the site of bone measured e.g. a weight-bearing exercise study has been accompanied by bone density measurements at the radius. Other studies have involved women over a wide age range and this may have obscured any benefits available in the immediate postmenopausal period compared with the very elderly (or vice versa).

Not surprisingly, findings from these studies therefore range from no apparent benefit at all (Blumenthal *et al.*, 1991, Smidt *et al.*, 1992) to marked increases in BMD. Dalsky *et al.*, (1988) noted an increase in lumbar BMD of 5.2% and 6.1% after 9 and 22 months respectively of walking, jogging and stair-climbing in 55 - 70 year old women. After 13 months of detraining, BMD had declined to 1.1% above baseline. Chow *et al.*, (1987) measured changes in bone mass of the trunk and upper thigh by neutron activation, expressed as the calcium index. After 1 year of aerobics and strength training 16 women, on average 7 years postmenopausal, had a significant increase in calcium index compared to non-exercising controls. In a 2 year longitudinal study of men and women over the age of 50 years, Michel *et al.*, (1991) found a high correlation between increasing exercise levels and bone density in those exercising at moderate levels but low levels of BMD at extremely high levels of exercise.

Others have shown that BMD may not increase with exercise in older women, but rates of bone loss may be slowed or halted (Rundgren *et al.*, 1984, Smith *et al.*, 1989, Rikli & McManis, 1990, Grove & Londeree, 1992, Revel *et al.*, 1993, Bassey & Ramsdale,

1995). This effect may be most marked in those women closest to the menopause (Martin & Notelovitz, 1993).

It therefore remains unclear whether exercise intervention in postmenopausal women is an appropriate form of treatment for established osteoporosis or whether indeed it can be advocated for the maintenance of bone density. Although cross-sectional and population studies support regular physical activity as a means of reducing osteoporosis, the volume and intensity of exercise required is still unknown. The effects of exercise on bone remodelling leading to net bone reduction may require the presence of premenopausal levels of sex hormones. Prospective studies of exercise intervention in postmenopausal women both on and off hormone replacement therapy would help answer this question.

Regular exercise may have other benefits in the elderly population at risk of osteoporotic fracture. Physical activity improves neuromuscular function and can reduce the rate of hip fracture from falling (Wickham et al, 1989). Exercise intervention programmes which improve balance and strength are also likely to reduce the rate of falling. This effect has been recently demonstrated by Wolf et al., (1996) as part of the Frailty and Injuries: Cooperative Studies of Intervention Techniques (FICSIT) Group. They achieved a 47.5% reduction in the risk of multiple falls in men and women over the age of 70 years who participated in a 15 week Tai Chi training programme. This alone is compelling evidence for maintaining physical activity in the elderly.

Section 9

Calcium intake and bone density in athletes

Bone mass is higher in children and adolescents with a high calcium intake (Chan, 1991; Sentipal *et al.*, 1991) and this may result in an increased bone mass in later life (Matkovic *et al.*, 1979). Work on healthy premenopausal women supports a role for

dietary calcium in the development of bone, particularly when associated with exercise (Kanders *et al.*, 1988; Halioua & Anderson, 1989). The synergistic effect of calcium and exercise on bone has also been demonstrated in animal models (Lanyon, 1986).

A positive linear relationship between trabecular BMD in the lumbar spine and calcium intake in athletes has been demonstrated by Wolman *et al.*, (1992) a finding that was independent of menstrual status and which has not been shown in other studies (Nelson *et al.*, 1986; Grimston *et al.*, 1990; Heinrich *et al.*, 1990). These differences may be due to methodology, particularly in assessment of calcium intake or statistical confounding by another risk factor. Alternatively the relationship between calcium intake and bone mineral content may not be linear. Kanders *et al.* (1988) showed a positive relationship between calcium intake and vertebral BMD in normal healthy eumenorrhoeic women but not above a daily intake of 800 - 1000 mg. The ability of an individual to adapt to a low calcium intake may be genetically determined (Krall 1995, Ferrari 1995).

Low calcium intakes have been reported in many athletes (Rucinski, 1989, Benson *et al.*, 1990, Pate *et al.*, 1990, Bergen-Cico & Short, 1992, Delistraty *et al.*, 1992, Frederick & Hawkins, 1992, Stensland & Sobal, 1992) particularly in amenorrhoeic women. Low oestrogen levels are associated with decreased intestinal absorption of calcium and increased urinary loss (Nordin & Heaney, 1990) so dietary calcium requirements may be even higher in amenorrhoeic athletes. Both Marcus *et al.* (1985) and Nelson *et al.* (1986) found that 55% of amenorrhoeic athletes failed to meet the recommended daily allowance (RDA) for calcium compared to 35 - 40% of cyclic women. Kaiserauer *et al.*, (1989) also noted a lower intake in amenorrhoeic compared to eumenorrhoeic runners (600 mg versus 1200 $mg.day^{-1}$, $P<0.05$). Yet it remains unclear how much of the variance in BMD in amenorrhoeic athletes is due to low calcium intake.

Section 10

Summary

Moderate regular physical activity appears to have beneficial effects on bone density at all ages though exercise intervention programmes in elderly women have not been able to demonstrate conclusive benefit. It remains unknown how much exercise and of what type should be recommended as optimal for the general population. High intensity training has advantages and disadvantages for skeletal metabolism and questions remain about long-term effects of menstrual irregularity on bone mineral. As yet there is very little information on the treatment of athletic amenorrhoea and its bony consequences.

CHAPTER 4

METHOD

1) Selection of Subjects

Female athletes were recruited to the study after advertising in running and veteran athlete magazines and by word of mouth. Women were recruited who fitted the following criteria:

a) Young Athletes

i) Aged 16 - 35 years

ii) Caucasian

iii) Non-smokers and in good health

iv) Currently training for and competing in middle and long-distance running events

v) Training at least three hours per week and running a minimum of 25 miles per week

vi) To have maintained this level of training for at least the last three years

vii) Not to have taken the oral contraceptive or other hormonal treatment in the last one year

b) Veteran Athletes

i) 40 years or over

ii) Caucasian

iii) Non-smokers and in good health

iv) Currently running at least 15 miles per week if a long-distance runner

v) Competing in veteran events and training for at least three hours a week if not a long-distance runner.

vi) To have maintained this level of training for at least the last three years

Exclusion criteria

Those who had a current or past history of respiratory disease, diabetes, metabolic bone disorders, rheumatoid arthritis, thyroid or parathyroid disease, malignancy, cardiac, renal or inflammatory bowel disease or who were taking oral or inhaled steroids were excluded because of their possible effects on bone metabolism. Possible recruits who had had anorexia nervosa or another eating disorder prior to starting training were not included. Menstrual irregularities were required to coincide with the onset of training or an increase in training load to be considered secondary to exercise.

All athletes gave informed consent as approved by the ethics committee of Northwick Park Hospital. Details of sporting activities, gynaecological history, past medical history, medications and smoking habit were obtained by questionnaire and direct enquiry:

i) **Gynaecological history**

age at menarche

menstrual cycle length

any previous or current irregularities in menstruation

dates of use of the oral contraceptive pill

number of pregnancies

details of the menopause

use of hormone replacement therapy

ii) **Sporting History**

age of starting sport of any kind

age of first training for current sport

other sports undertaken

details of previous and current methods of training including e.g. hours per week, miles per week, intensity

level of achievement

2) Grouping of Subjects

Athletes were categorised according to their menstrual history. The following definitions are used throughout;

eumenorrhoea	10 - 13 menstrual cycles per year
oligomenorrhoea	4 - 9 menstrual cycles per year
amenorrhoea	0 - 3 menstrual cycles per year excluding pregnancy

a) Young Athletes

i) Eumenorrhoeic (EU). Women who were currently and had always been eumenorrhoeic since menarche.

ii) Oligomenorrhoeic (OL). Women who had had 4 - 9 cycles per year for at least the last 18 months but had not had more than three months without a menstrual period.

iii) Amenorrhoeic (AM). Subjects who had had 0 - 3 cycles per year for at least the last 18 months.

b) Veteran Athletes

These subjects were first divided into pre and postmenopausal. They were defined as postmenopausal if they had not had a menstrual period for more than 12 months, associated with raised FSH levels +/- menopausal symptoms or a surgical menopause. Postmenopausal women who had had no gap between the onset of menopause and the commencement of Hormone Replacement Therapy (HRT) were considered as premenopausal.

Premenopausal veteran athletes only

Menstrual History

Subjects were required to have been eumenorrhoeic during the last one year. They were then categorised according to past menstrual history using the definitions in section 2a above.

i) Eumenorrhoeic (EU). Women who had always had 10 - 13 cycles per year since menarche.

ii) Non-eumenorrhoeic (Non-EU). Women who had a history of at least 2 years of either amenorrhoea or oligomenorrhoea which was considered secondary to their exercise habits.

Pre- and postmenopausal veteran athletes

Training History

Subjects were divided according to their current and past levels of physical activity. To be considered athletic in the past, subjects were required to have performed continuous aerobic exercise at least three times per week for a minimum of 20 minutes each time at 60 - 90% of their estimated maximum heart rate. This is the minimum recommendation for the development and maintenance of cardiorespiratory fitness as defined by the American College of Sports Medicine (1990).

Groups were:

i) All-timers (AT) - those who had consistently exercised since leaving school except when pregnant.

ii) Late-Starters (LS) - those who had been sedentary since leaving school but had taken up training after the age of 30 years

Thus the veteran athletes were categorised as follows:

Group 1 - Premenopausal

All-timer / always eumenorrhoeic (AT/EU)	Late-starter / always eumenorrhoeic (LS/EU)
All-timer / previous oligomenorrhoea (AT/non-EU)	Late-starter / previous oligomenorrhoea (LS/non-EU)

Group 2 - Postmenopausal

Group A	Group B
All-timer (AT)	Late-starter (LS)

3) Morphological characteristics

Height, weight, skin fold thicknesses and fat free mass were determined and from these values, body mass index and percentage body fat were calculated. All measurements were made by the same observer (author) each time, thus reducing inter-observer variation.

i) Height and weight

Using an Avery stadiometer and platform balance scale, height (in cms) and weight (in kg) were measured. From this, body mass index (BMI) was calculated from the following formula:

$BMI = W/H^2$ where W = weight in kg and H = height in metres

ii) Fat free mass

Fat free mass (FFM) was determined on each veteran athlete from a measurement of total body potassium (TBK) in mmol. The TBK measurements were made in a sodium iodide whole body counter using the technique described by Smith et al. (1979). The standard error on a value for TBK was approximately 4% and FFM was calculated from TBK on the assumption that there is an average of 60 mmol of potassuim per kg of FFM (Boddy et al , 1973) in healthy adult females:

$$TBK = 14.76(W) + 22.07(H) - 9.05(Age) - 1669$$

Thus, $FFM = TBK/60$

The values obtained for TBK were compared to the expected values for age of each individual, based on previously validated normal ranges for this NaI counter (Smith et al 1979).

Thus $\%TBK = TBKobs./TBKexp. \times 100$

where %TBK = % of mean of normal population of that age,

TBKobs = observed TBK in mmol

TBKexp = expected TBK in mmol for that age

iii) Percentage body fat

1) Skin fold thicknesses were measured at four sites using Harpenden skin fold calipers. The sites used were midbiceps, midtriceps, suprailiac and subscapular on the right side of the body. Three measurements were made to the nearest mm at each site and the mean of the three values was then taken. The sum of skin folds was the total

of the means from each site. Percentage body fat (%BF) was calculated according to the equation of Durnin and Wormesley (1974):

%BF = (495/(1.1576 - (0.0657 x log (sum of skinfolds)) - (0.00033 x age))) - 450

2) In the veteran athletes only, percentage body fat was also derived from the data obtained from the measurement of fat free mass as follows:

F = W - FFM, where F = fat in kg, W = weight in kg and FFM = fat free mass in kg
therefore %BF = F/W x 100

4) Bone mineral density

Bone Mineral Density (BMD) of the lumbar spine (L2-L4) and the left hip was measured in each subject by dual energy x-ray absorptiometry (DXA) using the Hologic QDR1000W densitometer and software version 6.1/4.26. This DXA system uses a narrow beam (1mm) of X-rays with two energy components of 70 and 140 KVp. The x-ray beam and detector unit are synchronised together and used to perform rectilinear scans of either the lumbar spine or hip region. Scanning time for the lumbar spine was about 8 mins and for the left hip, 7 mins. Daily calibration against a phantom in this laboratory has a long-term variation of 0.1%. In vivo reproducibility of DXA is about 1% and effective dose equivalent for females is estimated at 6.4uSv (Pye et al 1990). Scan analysis was performed by technicians with daily experience in DXA analysis and who were blinded to the menstrual or activity status of the athlete.

i) Lumbar spine

The subject lies supine on the table with the legs elevated on a foam block and hips flexed to 45^{o}. With the patient in this position, a rectilinear scan (12.5cm wide x

20.0cm long) was performed of the lumbar spine. Subsequently, a region of interest 109 pixels* wide and of such length to include L2 - L4 was selected.

*(a pixel is the picture element of a matrix display whose size varies according to the size of the display, in this case 1 pixel = 0.0965 x size of display)

ii) Left Hip

The subject lies supine on the table with the left foot placed in a femur repositioning aid which places the femur in 25^{o} of anteversion. In this position a rectilinear scan (approximately 15cm x 15cm) was performed of the left hip region. Then for the purposes of data analysis, a region of interest was selected that included tha entire femoral head and the greater and lesser trochanters. BMD values ($g.cm^{-2}$) were calculated for the following regions of the proximal femur using Hologic software. The following descriptions of the three areas of interest of the proximal hip are taken from the Hologic $QDR^{-1}000$ Operators Manual Oct. 1989.

Femoral Neck - the default size of the femoral neck is defined as a rectangular region 1.5cm wide and 6cm or less long where the bottom line of the neck region is centered 0.75cm below the narrowest area of the femoral neck.

Ward's Triangle - this is the area of the femoral neck deemed to be highest in trabecular bone and is therefore an area of initial bone loss in osteoporosis. Ward's Triangle is determined automatically by searching for an area of minimum density in the femur. Initially the analysis compares an area of 11 x 11 pixels (1cm x 1cm) within a box of 27 x 35 pixels (2.7cm x 3.5cm) centred on the lower end of the femoral neck box (see diag.2). Some patients (especially young patients) do not have a defined area of minimum density in the femoral neck and analysis of these patients may not give a true density for Wards triangle.

Trochanteric Region - this is a triangular region whose boundaries are defined as the lateral edge of the femoral neck region and a line connecting the midpoint of the femoral midline to the point where the edge of the femur changes curvature below the trochanter.

Expression of bone mineral density

1) Bone mineral density is expressed initially as $g.cm^{-2}$. Although this is not strictly a density it is the form used by manufacturers of DXA machines and most investigators and has therefore been used in this thesis for purposes of comparison and consistency.

2) Using the Hologic database, each athlete was compared to the peak bone mass for that region of interest (T-score). The Hologic database is derived from measurements on 747 American women judged to be free from disorders or medication likely to influence bone metabolism. Peak BMD (PBMD) is deemed to occur at 22.0 years for the femoral neck and 30 .0 years for the trochanteric region and lumbar spine.

3) During the course of the study, it became apparent that there are differences between the reference databases of the various DXA machines available e.g. Hologic, Lunar, and concern has arisen that the Hologic reference database for neck of femur deviates from those derived from large samples of normal subjects (Genant et al., 1994, Laskey et al., 1992, Pocock et al., 1992). Some of the work of the 'Concerted Action of the European Community's COMAC-BME' programme was undertaken in the same laboratory and it was possible to use this newly developed reference range, instead of the Hologic reference range, for age-matched data.

The work by 'COMAC' has resulted in a European reference range for bone density for both pre and postmenopausal women. This group measured proximal femur and

lumbar spine bone densities of 855 women randomly selected from the normal populations of 12 clinical centres in 8 countries. The three UK centres were Harrow, Manchester and Aberdeen. Statistical analysis between machines and centres has enabled the development of equations that now allow direct comparison with the mean of the age-matched European reference range (Z-score) (Dequeker et. al., 1995, Pearson et. al., 1995 a and b).

To enable this comparison to be made, measured BMD ($g.cm^{-2}$) is first converted to a specified density (X) using the equation $X = 3.042 - (1/0.359 \times \ln (2.98 - g.cm^{-2}))$. This makes allowance for differences between the various machines used in the different centres. The new specified density allows comparison with the mean of the European population data using the following equations:

Premenopausal women

Neck of Femur	Z = LN(X) - 0.0275 + (0.003 x age)/0.1428
Trochanteric	Z = LN(X) + 0.2836/0.14977
L2 - L4	Z = LN(X) - 0.1803/0.1253

Postmenopausal women

Neck	Z = LN(X) + 0.14518 + (0.008324 x YSM) / 0.15544
Trochanteric region	Z = X - 0.74811 + (0.004958 x YSM / 0.11736
L2 - L4	Z = LN(X) - 0.08127 + (0.00729 x YSM / (0.12813 + (0.0038 x YSM))

where Z = Z-score

X = specified density

LN = natural logarithm

YSM = years since the menopause

T-scores and Z-scores are expressed as standard deviations (e.g. mean+1SD = +1).

5) Physiological characteristics

Grip strength, isokinetic back strength and maximal uptake and utilisation of oxygen (VO_2max) were determined as measures of fitness levels and strength. Each test was explained, demonstrated and assessed by the same operator (author) throughout.

i) Grip Strength

Grip strength in Kgf was determined in each hand of the veteran athletes using a Takei grip dynamometer. Using a standardised method, grip strength was measured three times in each hand with 30 seconds rest between each attempt. The best attempt from the dominant and non-dominant hand was taken.

ii) Isokinetic Back Strength

Isokinetic back strength was measured using a Loredan Lido™ Active dynamometer. The Lido dynamometer measures force applied to a peripheral attachment moving in an arc about a fulcrum at a specified speed. To isolate trunk movements, the subject is placed in a sitting position with the upper body clamped between two pads. The pelvis is immobilised by a seat belt and the lower limbs held firm by a strap. The subject is then able to move the equipment, using trunk flexor and extensor muscles only, through a specified arc (30^o extension to 30^o flexion) with the hip at the point of the fulcrum. The fulcrum will rotate at a set speed and maximum power is achieved by asking the subject to push as hard and as fast as possible against the pads. Peak Torque and Peak Torque to Body Weight ratio of both trunk flexion and extension were measured at two different fulcrum speeds ($60^o.sec^{-1}$ and $180^o.sec^{-1}$). Each subject was fully habituated to the equipment and the test before commencing. Three consecutive efforts were performed and the peak torque taken as the maximum force applied in each direction at any stage of the test. Verbal encouragement was given throughout.

iii) Maximal Uptake and Utilisation of Oxygen (VO_2max)

This test gives an indication of the aerobic fitness of the athlete and was performed on a Powerjog treadmill using the Jaeger EOS Sprint on-line gas analysis system or the Cosmed K2 portable on-line gas analysis system calibrated to the Jaeger. Constant readout of the electrocardiograph (ECG) was obtained from three skin contact electrodes placed on the chest. The subject was allowed time to acquaint themselves with the treadmill before starting the test. After a five minute warm-up period at a suitable speed, individualised for each subject, the speed of the treadmill was increased by 1.1kph each minute. When the respiratory quotient (RQ)* rose over 1.0, the speed was maintained and the gradient of the treadmill increased by 1% each minute. The test was continued to volitional exhaustion and verbal encouragement was given throughout. Total time for the test was between 8 mins. and 15 mins.(in accordance with the British Association of Sports Sciences, BASS, recommendations).

Criteria for reaching VO_2max were:

1) No further increase in VO_2 despite increase in workload
2) RQ of greater than 1.10
3) Max heart rate (MHR) reached based on predicted value for age (predicted MHR = 220 - age)

* Respiratory Quotient is the ratio of carbon dioxide breathed out to oxygen usage. When fat is burned for energy, the RQ is 0.7 and when carbohydrates are utilised, the RQ is 1.0. An RQ of greaater than 1.10 indicates anaerobic metabolism.

6) Biochemical Tests

Blood and urinary tests for renal function and bone turnover were as follows:

a) Serum calcium, phosphate, alkaline phosphatase, albumin and osteocalcin

b) Serum sodium, potassium and creatinine

c) Urinary hydroxyproline, calcium, creatinine

Tests for hormonal status were:

d) Serum oestradiol

e) Follicle Stimulating Hormone (FSH)

If appropriate, pregnancy was excluded prior to measurement of bone density:

f) bHCG (human chorionic gonadotrophin) on early morning specimen of urine

a) Blood was collected for serum calcium (Ca), phosphate (PO_4), alkaline phosphatase (AlkP) and albumin (Alb) using standard venepuncture techniques with minimal use of the tourniquet. The blood samples were taken immediately to the Northwick Park Hospital laboratories for analysis. Samples were separated and analysed using the KODAK EKTACHEM colorimetric slides for Ca, PO_4 and Alb and the KODAK EKTACHEM multiple-point rate test for AlkP.

Blood for osteocalcin assay was collected as above, allowed to clot at room temperature and then centrifuged at 2400rpm for ten minutes. It was frozen at -20^{o}c and analysed in batches of 50 using the Incstar ^{125}I radioimmunoassay (Incstar Corporation, Stillwater, Minnesota, USA). This assay was kindly performed by the Clinical Research Centre laboratories at Northwick Park Hospital.

b) Serum collected as above was analysed for sodium (Na) and potassium (K) using the KODAK EKTACHEM potentiometric slides and creatinine (creat) was measured by the KODAK EKTACHEM blank-corrected colorimetric test in the Northwick Park Hospital Laboratories..

c) A fasting early morning urine specimen was collected on the day of attendance at the BOMC following a 24 hour gelatine free diet. The sample was separated into two aliquots:

i) analysis of urinary calcium (UrCa) and urinary creatinine (UrCreat) was performed, using the KODAK EKTACHEM colorimetric tests, by the Northwick Park Hospital laboratories

ii) the second aliquot was frozen at -20^{o}c and analysed in batches of 20 for urinary hydroxyproline (UrHP), using a spectrophotometric method developed and performed by the Bone Diseases Research Group in the Clinical Research Centre laboratories.

From these results, UrHP:UrCreat and UrCa: UrCreat ratios were calculated.

d) Using the above venepuncture technique, blood was collected for oestradiol and FSH estimation. It was separated by centrifuge at 2400 rpm for 10 minutes and stored at -20^{o}c. Oestradiol was measured using the DPC Coat-a-Count ^{125}I radioimmunoassay (Diagnostic Products Corporation, Los Angeles) in batches of 30 samples, thus reducing the interassay variation.

FSH was measured using the Abbott Laboratories Microparticle Enzyme Immunoassay, also in batches of 30.

e) The measurement of Lumbar Spine bone density using DXA exposes the female gonads to small doses of radiation and exclusion of pregnancy was therefore required in some subjects prior to this procedure. ßHCG was estimated using the Organon Neo-Planotest 200, a direct latex agglutionation slide test, on an early morning specimen of urine.

7) Dietary Calcium Intake

Current and past dietary calcium intake was assessed using the Calquest 2.1 Dietary Questionnaire (Nelson et al., 1988). Responses were recorded immediately onto the

computer version of the questionnaire which assessed the daily intake in $mg.day^{-1}$. To assist in standardisation of portion sizes, appropriate photographs were used. This questionnaire has previously been validated against five-day and seven-day weighed dietary inventories with reported correlation coefficients of r=0.76 and r=0.69 respectively.

8) Eating Disorders

Eating habits were assessed in the 16 - 35 year age group only. The Eating Attitudes Test (EAT26) and the Bulimia Investigatory Test Edinburgh (BITE) questionnaires were used which have been validated for anorexia (Garner et al., 1982) and bulimia (Henderson et al., 1987) respectively. A score of >30 on the EAT26 score represents a high likelihood of anorexia and a score of 15 - 30 represents a sub-clinical group with disturbed eating habits and anorectic attitudes. The EAT26 is also divided into three further factors: Factor 1 is related to a preoccupation with being thinner, Factor II relates to bulimia and food preoccupation and Factor 3 relates to self-control of eating. These subdivisions was also analyzed in our sample. In the BITE questionnaire, a 'symptom scale score' of >20 represents highly disordered eating and the presence of binge-eating and a score of 10 - 19 reflects a group with unusual or disturbed eating habits (subclinical bulimia nervosa). A score of <10 is considered normal. A 'severity scale' on the BITE questionnaire assesses degree of any binge-eating and purging.

CHAPTER 5

DETERMINANTS OF BONE MINERAL DENSITY IN FEMALE MIDDLE AND LONG-DISTANCE RUNNERS

Abstract

Objectives

To determine whether intensive weight-bearing exercise can offset bone mineral loss associated with secondary amenorrhoea due to athleticism.

To assess morphological and nutritional parameters affecting bone mineral density in highly trained athletes

Methods

50 national standard middle and long-distance female runners aged 16 to 35 years (mean 26.3 years) were studied. Twenty-four had amenorrhoea (AM) and 9 had oligomenorrhoea (OL), for at least the last 1.5 years. 17 had always been eumenorrhoeic (EU). BMD of the proximal femur and lumbar spine was measured by Dual Energy X-ray Absorptiometry and compared with both peak bone mass and with age-matched European reference data.

Results

BMD was low in the AM group at all sites measured but high in the proximal femur in EU athletes. In the AM group, height and weight were independent predictors of BMD. Eating disorders were more common in this group and correlated well with low body weight and body mass index.

Conclusions

Eumenorrhoeic runners have high peak bone mass in the proximal femur but in amenorrhoeic athletes, despite intensive weight-bearing exercise, peak bone mass is lower than appropriate in the proximal femur as well as in the lumbar spine.

Introduction

Physical activity has been shown to have site-specific effects on bone mineral density (BMD). For instance, in tennis players, bone density is higher in the dominant arm than the non-dominant arm (Dalen *et al.*, 1985, Pirnay *et al.*, 1987, Haapasalo *et al.*, 1994) and rowers have high lumbar bone density associated with their greater back strength (Wolman *et al.*, 1990). However, intensive exercise can predispose to secondary amenorrhoea and this condition is associated with low bone density, particularly in the lumbar spine (Drinkwater *et al.*, 1984, Marcus *et al.*, 1985) Concern has arisen that these athletes may be at risk of early osteoporosis and fracture. Most work in runners has assessed BMD of the lumbar spine and non weight-bearing areas such as the radius but there is much less data available on the proximal femur, an important site of osteroporotic fracture in later life. It seems possible that femoral bone density may be maintained despite amenorrhoea in runners and that they may not be at increased risk of later hip fracture.

The aim of this study was to investigate site-specific effects of running on BMD and to determine whether menstrual dysfunction has significant effects on femoral BMD.

Methods

50 national and international standard caucasian runners aged 16 - 35 years were recruited by advertisements in running magazines according to the criteria in Chapter 4. Seventeen women were considered eumenorrhoeic (EU), 9 had oligomenorrhoea (OL) and 24 were amenorrhoeic (AM).

Questionnaires gave menstrual, obstetric and training histories. Eating habits were assessed by the EAT26 and BITE questionnaires. A general medical examination confirmed fitness to undertake the tests and informed consent was obtained.

Body Composition, Physiological Characteristics and Dietary Calcium Intake

As previously described in Chapter 2 , the following measurements were made:

i) weight and height

ii) body mass index (BMI; weight/height^2)

iii) percentage body fat using the four-site skinfold thickness method

iv) isokinetic back strength

v) dietary calcium intake

Bone mineral density

Bone Mineral Density (BMD) in g.cm^{-2} of the lumbar vertebral bodies (L2-L4) and the left hip (neck of femur, FN, and trochanteric region, FT) was measured in each subject by dual energy x-ray absorptiometry (DXA), as described in Chapter 4. Scan analysis was performed by technicians who were blinded to the menstrual status of the athlete. The BMD value of each region of interest was compared to the Hologic reference population at peak bone mass (T-score) and to the age-matched European population data (Z-score) as previously described.

T-scores and Z-scores were expressed as standard deviations (e.g. mean + 1SD = +1).

Statistical analysis

The statistical package used was Microsoft Excel 5.0. Data were analysed by a single factor analysis of variance across menstrual groups followed by a two-tailed students 't' test for between-group differences. Although small numbers were involved in some of the comparisons, the data were considered to be normally distributed and parametric tests were felt to be appropriate. Comparison of T-scores and Z-scores against the reference range was performed using an independent 't' test. Relationships amongst variables were analysed using either simple correlation or linear regression with BMD as the dependent variable. Further parameters were then added to the

model in a forward selection multiple regression. Significance was accepted throughout at $p<0.05$.

RESULTS

1) Demographic, menstrual and training characteristics

See Tables 1 - 3. The three groups were similar in age and height. Amenorrhoeic athletes were lighter and had a lower body mass index than the EU athletes. They also had lower body fat than both the EU and OL women. As expected the AM athletes had had fewer years of eumenorrhoea during their lifetime but there was no significant difference between the duration of menstrual disturbance between the AM and OL groups. Age at menarche was similar across groups.

The average age of commencement of training for running was 20 years across the cohort with no differences between the groups. The AM athletes ran slightly more miles per week but this difference was not significant and indeed the mean VO_2max was remarkably similar across groups. Seventy-one per cent, 44% and 35% of AM, OL and EU athletes respectively ran or had run for Great Britain, with the remainder competing at top National level.

2) Eating habits

Eating habits were significantly different between the AM and EU groups (Table 4 and Graph 1). In the AM group, 1 athlete had an EAT26 score of >30 (clinical anorexia) and 8 had scores of 15 - 30 (disturbed eating). One subject on treatment for anorexia did not complete her questionnaire and one subject with independently verified anorexia and bulimia did not fully complete her questionnaire. One EU subject did not return her questionnaire, reason unknown. The percentage of each menstrual group in each category of EAT26 score are shown in Graph 1. The BITE symptom score revealed disturbed eating (score of 10 - 19) in 45% (AM), 33% (OL) and 25% (EU)

athletes. One OL athlete had severe binge-eating. The EAT26, Factor 1 and Factor 3 scores were all negatively correlated with weight (r=-0.371, -0.32 and -0.44 respectively, $p<0.02$). The BITE score was not correlated with weight.

3) Calcium intake

Mean daily calcium intake was slightly higher in the EU group but this did not achieve significance (Table 5). A large number of athletes consumed less than 600mg per day (current recommended daily allowance); 25% (AM), 55.6% (OL), 17.6% (EU). Linear regression of BMD with calcium intake showed a relationship ($p=0.058$) with trochanteric BMD but this was eradicated when menstrual status was added to the model. In addition there was no difference in BMD between those who consumed less than or more than either 600mg/day or 1000mg/day.

4) Bone Mineral Density

Data analysed using $g.cm^{-2}$

BMD values in $g.cm^{-2}$ and between group analysis are shown in Table 6. There was an approximate linear relationship between menstrual group and BMD with significantly lower BMD in those with the greatest menstrual dysfunction. Bone density was 16.5%, 19.5% and 15.6% lower in the AM group compared to the EU group in FN, FT and LS regions respectively. Similarly, BMD was 12.4%, 11.1% and 10.1% lower in the OL group compared to the EU group.

Regression Analysis

Results of linear regression of BMD as the dependent variable with various possible independent variables are shown in Appendix 1, Table 1. Many of these were felt to have colinearity with menstrual status and further multiple regression was therefore performed with menstrual status as the main independent variable. Results of this further step are shown in tables 2, 3, 4.

Femoral neck

When adjusted for menstrual status, previously noted correlations were no longer significant.

Trochanteric region

Age of the athlete and age at which the athlete started training (age began) were negatively associated with BMD after adjustment for menstrual status. However, age began was highly correlated with the age of the athlete (r=0.78) and one possible explanation is that the greater number of years spent training, the greater the accrual of bone mineral. After adjustment for menstrual group, the greater the number of years of eumenorrhoea, the greater the BMD at this region.

Lumbar spine

Height also contributed to the variance, even when menstrual status was accounted for.

Linear regression by menstrual group

To determine within group correlations, regression analysis was performed separately for each group for height and weight. Results of this are shown in Appendix 1, Table 5. Bone density was consistently correlated with height in all areas in the AM group but not in other groups. Similarly, weight was a predictor of bone density in the AM group but only in the femur and lumbar spine.

Comparison with the age-matched European reference range

Comparison of BMD with European data (Z-scores) is shown in Table 7, Graph 2. In comparison to the expected mean for the groups, the AM group had significantly lower Z-scores at all regions, and the EU athletes had higher BMD at femoral regions. Values in the lumbar spine for the OL athletes were significantly lower than expected.

Between group analysis revealed the AM and OL athletes to have significantly lower Z-scores in all regions than the EU athletes ($p<0.001$, $p<0.01$ respectively). Although the Z-scores were higher in the OL group compared to the AM athletes this did not reach significance.

Linear regression, using the whole cohort, revealed very similar results to those obtained when using the unadjusted BMD $g.cm^{-2}$. When menstrual status was added to the model only three relationships remained significant: low FT BMD was associated with later age at which training began and fewer years of eumenorrhoa ($p<0.05$ each) and LS BMD was associated with height ($p<0.01$). The AM athletes were significantly lighter than the other athletes but when weight was accounted for in the model, menstrual status remained a significant variable ($p<0.0001$). There were no significant relationships between BMD (any region) and any of the other measured parameters (Appendix 1, tables 6,7,8)

The groups were then considered separately to determine within group correlation of BMD with height, weight, BMI and % body fat. In the AM group, BMD was significantly correlated with height at all regions (LS $p=0.001$, FN $p<0.001$, FT $p<0.05$) and weight at LS ($p<0.02$) and FN ($p<0.05$). These associations were not evident in the OL and EU groups. Within the amenorrhoea group alone, years of amenorrhoea was not correlated with BMD.

Comparison with the Hologic reference range for peak bone mass

Comparison of BMD with peak bone mass (T-score) is shown in Table 8, Graph 3. T-scores were significantly low at all regions in the AM group and in the spine in the OL group. EU athletes had high T-scores in the femoral regions.

5) Biochemical parameters

The mean values for biochemistry are shown in Table 9. Serum data were unavailable for one AM and one OL athlete due to laboratory error. An appropriate urine sample was not provided by three AM and one OL athlete. Serum phosphate was much higher in the AM group compared to OL and oestrogen levels were lower in AM athletes than the other 2 groups. This was only significant when compared to EU athletes, probably because of small numbers in the OL group. Mean prolactin levels were high in the EU group. These results were skewed by 3 athletes who had very high results (>600 IU/ml, normal range <400 IU/ml). Subsequent investigation revealed no abnormality. Osteocalcin levels were highest in the EU group ($p<0.005$).

Table 1

Demographic Variables of Athletes by Menstrual Group.

Mean (Standard Deviation)

Group	**AM** (n=24)	**OL** (n=9)	**EU** (n=17)	**ANOVA**	**P-value**
Age (years)	27.21 (5.0)	25.44 (6.7)	26.29 (6.4)	$F_{2,47} = 0.33$	0.72
Height (cm)	164.6 (4.5)	163.7 (4.3)	165.9 (4.5)	$F_{2,47} = 0.84$	0.44
Weight (kg)	51.3 (5.1) **	53.9 (5.6)	57.5 (5.4)	$F_{2,47} = 7.05$	0.002
Body Fat (%)	17.6 (4.5) *	21.3 (7.3) ϕ	21.0 (1.8)	$F_{2,47} = 5.37$	<0.01
BMI (kg/m^2)	18.8 (1.8) **	20.1 (2.1)	20.9 (1.3)	$F_{2,47} = 7.74$	<0.002

Menstrual groups: AM = 0 - 3 menses per year, OL = 4 - 9 menses per year, EU = 10 - 13 menses per year.

Student's 't'-test : *p<0.01, **p<0.005 for AM vs. EU;

ϕ p<0.05 for AM vs. OL

Table 2

Menstrual Characteristics of Athletes by Menstrual Group

Mean (Standard Deviation)

Group	**AM** (n=24)	**OL** (n=9)	**EU** (n=17)	**ANOVA**	**P-value**
Age at Menarche (years)	13.8 (1.3)	14.1 (1.8)	14.1 (2.2)	$F_{2,47} = 0.16$	0.85
Years of Eumenorrhoea	6.4 (4.8)**	7.1 (5.2)	11.7 (6.6)	$F_{2,47} = 4.87$	<0.02
Years of OL/AM	7.0 (3.7)	4.9 (3.8)	-	$F_{1,31} = 2.25$	0.14

Menstrual groups: AM = 0 - 3 menses per year, OL = 4 - 9 menses per year, EU = 10 - 13 menses per year.

Student's 't'-test : **p<0.005 for AM vs. EU

Table 3

Training Characteristics by Menstrual Group

Mean (Standard Deviation)

Group	**AM** (n=24)	**OL** (n=9)	**EU** (n=17)	**ANOVA**	**P-value**
Miles run per Week	54.3 (20.5)	47.3 (14.25)	44.4 (16.3)	$F_{2,47} = 1.56$	0.22
VO2max ($ml.kg.^{-1}min.^{-1}$)	58.9 (7.3) (n=22)	57.9 (7.6)	58.2 (5.5) (n=16)	$F_{2,44} = 0.04$	0.96
Age began Training (years)	20.2 (5.8)	20.7 (5.5)	19.6 (6.4)	$F_{2,47} = 0.11$	0.90

Menstrual groups: AM = 0 - 3 menses per year, OL = 4 - 9 menses per year, EU = 10 - 13 menses per year.

Table 4

Total EAT26 and BITE Scores.

Mean (Standard Deviation)

Group	**AM** (n=22)	**OL** (n=9)	**EU** (n=16)
EAT26	12.3 (9.4) **	10.4 (8.6)	5.6 (6.0)
Factor 1	7.7 (6.3) *	7.4 (5.8)	3.8 (3.8)
BITE 'symptom score'	7.6 (3.7)	9.2 (6.1)	5.4 (3.7)

Menstrual groups: AM = 0 - 3 menses per year, OL = 4 - 9 menses per year, EU = 10 - 13 menses per year.

2 athletes in AM group and 1 athlete in EU group failed to return questionnaires

* $p<0.05$, ** $p<0.02$ for AM vs.EU

Table 5

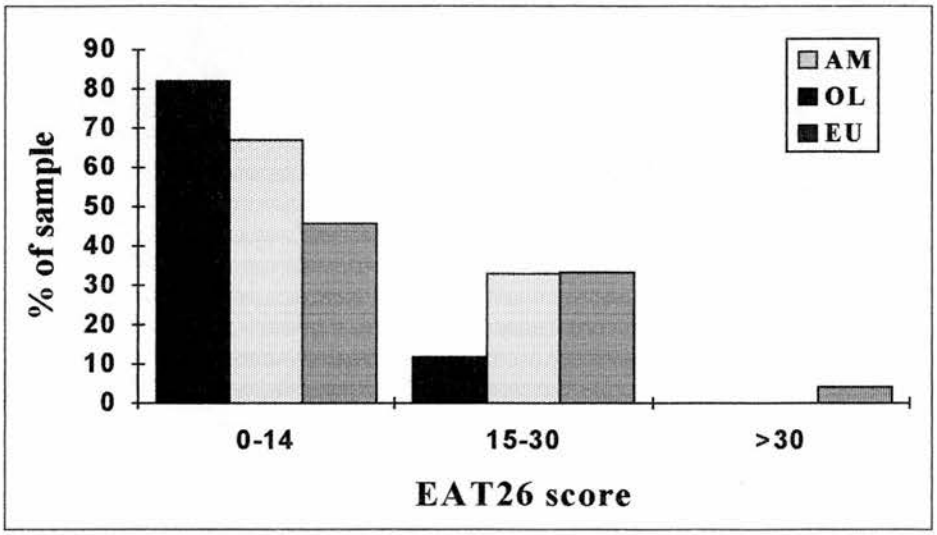
Dietary Calcium Intake by Menstrual Group

Mean (Standard Deviation)

Group	**AM** (n=24)	**OL** (n=9)	**EU** (n=17)
Dietary Calcium Intake ($mg.day^{-1}$)	829.1 (387.8)	807.7 (484.6)	1055.7 (440.6)

No significant differences in calcium intake between groups

EAT26 Score in Female Athletes



Graph 1

Attitudes towards eating assessed by EAT26 questionnaire. Scores of 0-14 represent normal eating, 15-30 represents disordered eating with anorectic attitudes, >30 indicates clinical anorexia.

AM: amenorrhoeic athletes (n=22)
OL: oligomenorrhoeic athletes (n=9)
EU: eumenorrhoeic athletes (n=16)

Table 6

BMD ($g.cm^{-2}$) by menstrual group.

Mean (Standard Deviation)

Group	FN	FT	LS
AM (n=24)	0.81 (0.08)***	0.66 (0.11)***	0.92 (0.10)***
OL (n=9)	0.85 (0.12)*	0.73 (0.07)***	0.98 (0.07)**
EU (n=17)	0.97 (0.11)	0.82 (0.07)	1.09 (0.10)

FN = Neck of Femur, FT = Trochanteric Region, LS = Lumbar Spine, L2-L4

Students 't' test:
**** $p< 0.0001$ for AM vs. EU
*** $p< 0.005$ for OL vs. EU
** $p< 0.01$ for OL vs EU
* $p< 0.02$ for OL vs EU

No significant differences between AM vs. OL

Table 7

Comparison of BMD with age-matched mean of European reference population

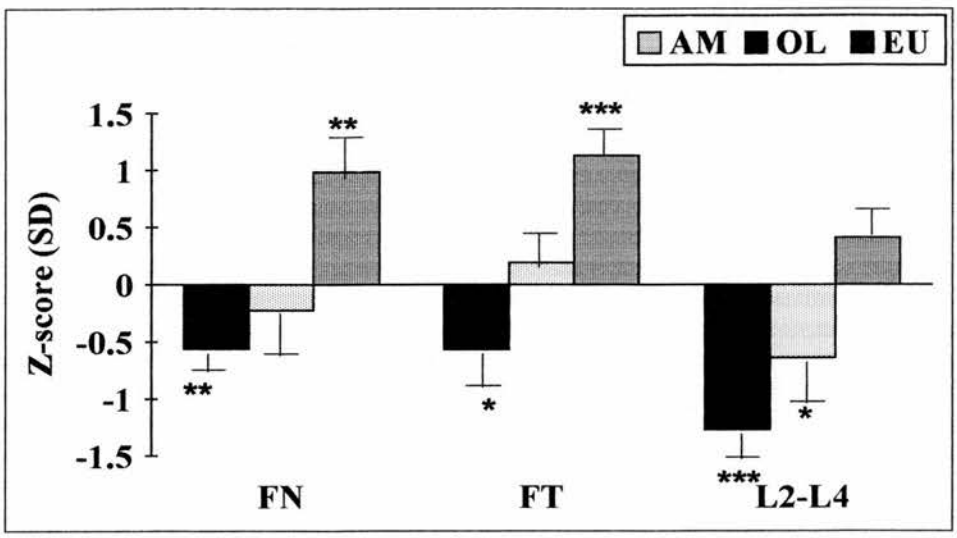
Mean Z-Score (SD of Z-Score)

Group	FN	FT	LS
AM (n=24)	-0.57 (0.74) **	-0.57 (1.19) *	-1.27 (1.0) ***
OL (n=9)	-0.23 (1.14)	+0.19 (0.6)	-0.64 (0.7) *
EU (n=17)	+0.98 (1.0) **	+1.14 (0.69) ***	+0.41 (0.92)

Bone mineral density was compared with European population data using equations prepared by Dequeker *et al.*, 1995 Pearson *et al.*, 1995. Z-score = SDs above or below the population mean.

* P<0.05, ** P<0.005, *** P<0.001

Bone Mineral Density (Z-score) of Proximal Femur and Lumbar Spine in Female Athletes



Graph 2

Mean (+/- SE) of bone mineral density of middle and long-distance runners compared to the age-matched European reference range (Z-score). Z-score given in standard deviations above or below the mean.
FN: neck of Femur, FT: trochanteric region, L2-L4: lumbar spine.

t-test compares each group with the European range:
*p<0.05, **p<0.005, ***p<0.001

AM: amenorrhoeic athletes (n=24)
OL: oligomenorrhoeic athletes (n=9)
EU: eumenorrhoeic athletes (n=17)

Table 8

Comparison of BMD with peak bone mass of Hologic reference population

Mean T-score (Standard Deviation of T-score)

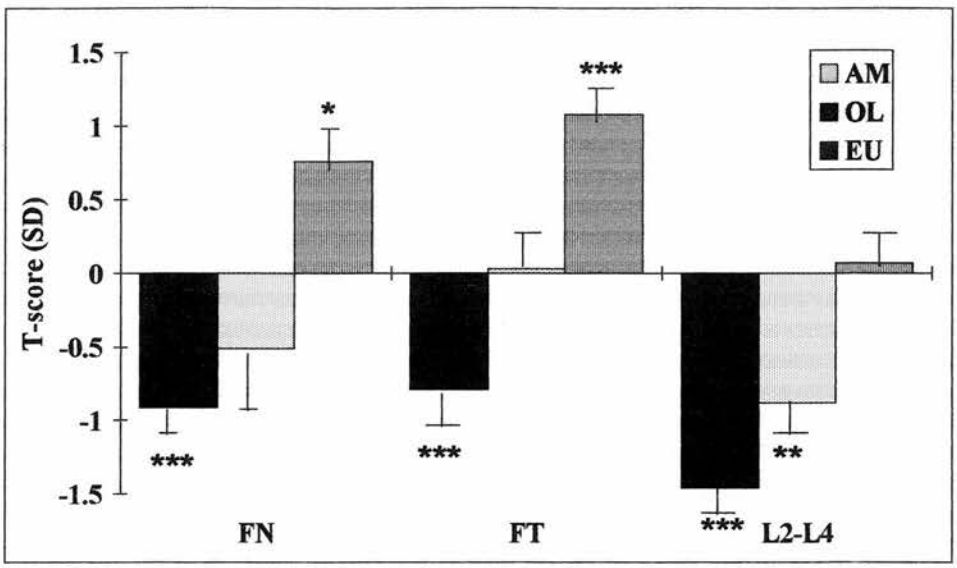
Group	FN	FT	LS
AM (n=24)	-0.91 (0.74)***	-0.79 (0.96)***	-1.46 (0.88)***
OL (n=9)	-0.51 (1.22)	+0.03 (0.65)	-0.88 (0.66)**
EU (n=17)	+0.76 (1.14)*	+1.08 (0.80)***	+0.07 (0.86)

FN = Neck of Femur, FT = Trochanteric region, LS = Lumbar Spine L2-L4

T-score = SDs above or below the mean peak bone mass of American population at age 22 years (FN) and 30 years (FT and LS) using Hologic reference database (T-score).

p values: *<0.02, ** <0.01, *** <0.001

Bone Mineral Density (T-score) of Proximal Femur and Lumbar Spine in Female Athletes



Graph 3

Mean (+/- SE) of bone mineral density of middle and long-distance runners compared to the Hologic reference range. T-score = standard deviations above or below the mean peak bone mass of the American population at age 22 years (FN) and 30 years (FT and L2-L4).
FN: neck of Femur, FT: trochanteric region, L2-L4: lumbar spine.

t-test compares each group with the Hologic range:
*$p<0.02$, **$p<0.01$, ***$p<0.001$

AM: amenorrhoeic athletes (n=24)
OL: oligomenorrhoeic athletes (n=9)
EU: eumenorrhoeic athletes (n=17)

Table 9

Biochemical Parameters of Bone Turnover and Pituitary Function

Mean (Standard Deviation)

Group	**AM (n=23)**	**OL (n=8)**	**EU (n=17)**
Serum Parameters			
Calcium ($mmol.l^{-1}$)	2.32 (0.07)	2.31 (0.04)	2.31 (0.09)
Phosphate ($mmol.l^{-1}$)	1.26 (0.12)ϕ	1.10 (0.15)	1.18 (0.16)
Alkaline Phosphatase ($mmol.l^{-1}$)	62.74 (15.70)	56.25 (11.71)	62.29 (24.93)
Oestrogen ($pg.ml^{-1}$)	161.3 (173.1)*	331.4 (350.6)	325.8 (229.7)
FSH ($mIU.l^{-1}$)	5.50 (2.57)	5.09 (2.68)	5.10 (2.61)
PRL ($IU.l^{-1}$)	177.4 (90.7)	170.6 (84.7)	350.9 (283.9)*
Osteocalcin ($ng.ml^{-1}$)	2.45 (0.93)	2.63 (1.24)	3.52 (1.14)*
Urinary Parameters	**(n=21)**	**(n=8)**	**(n=17)**
Ca:creat	0.47(0.35)	0.29 (0.20)	0.34 (0.23)
OHPr:creat	38.7 (68.7)	21.1 (11.8)	21.4 (12.8)

FSH = Follicle Stimulating Hormone

PRL = Prolactin

Ca:creat = Urinary calcium : creatinine

OHPr:creat = Urinary hydroxyproline : creatinine

Students 't' test: ϕ p=0.005 AM vs.OL

* p<0.02 AM vs EU, ** p<0.005 AM vs EU

DISCUSSION

1) The effect of menstrual history on BMD

The main focus of this study was to shed further light on the interplay between low bone density due to athletic amenorrhoea and the beneficial effects of mechanical stress at appropriate bone sites. The results for lumbar BMD support previous findings that BMD is markedly low in amenorrhoeic runners. The relationship between menstrual history and BMD was virtually linear between the groups. This may have been further highlighted if data for lifetime number of menstrual cycles had been used rather than the less sensitive categorisation used here. Nevertheless, the results are very similar to those obtained by Drinkwater *et al.,* (1990) who showed a linear relationship between menstrual history and lumbar BMD in 97 active women. In the Drinkwater study those women with the severest menstrual dysfunction had BMD 17% lower than those who had always been eumenorrhoeic and in this study the difference was 15.6%.

Highly trained middle and long-distance runners perform very little training other than running which exerts minimal direct mechanical stress on the spine. In addition, the lumbar spine is high in trabecular bone which is known to lose bone mineral early in low oestrogen states. It is therefore not surprising that if detrimental effects of low oestrogen levels are to be seen in such athletes, they will occur at this site.

What is surprising is the extent to which BMD is affected in these young women. The emergence of the new European population reference range (Pearson *et al.,* 1995a,b) has enabled comparison of the BMD of this small sample with a large number of age-matched women. The finding that, on average, British top-class amenorrhoeic runners have lumbar BMD that is 1.3 standard deviations below the population mean, with 54% having BMD >1.5 SD's lower gives concern for the future. At present there is no method available to predict lifetime risk of vertebral fracture from peak bone density

but if these women remain in the lowest quartile of BMD until the menopause, Kroger et. al., (1995) estimate that they may be at three times greater risk of fracture than those in the highest quartile.

Weight-bearing exercise is associated with high BMD in weight-bearing skeletal sites in eumenorrhoeic athletes (Heinrich *et al.*, 1990, Risser *et al.*, 1990, Wolman *et al.*, 1991, Slemenda *et al.*, 1993). It would be expected therefore that the muscular stresses exerted on the femur during running, combined with ground reaction forces, could offset bone mineral loss associated with amenorrhoea, although this study has clearly shown that a linear relationship also exists between degree of menstrual irregularity and femoral BMD at both the trochanteric region and the femoral neck. Whereas lumbar BMD ($g.cm^{-2}$) was 15.6% lower in the amenorrhoeic athletes compared to the eumenorrhoeic runners, BMD ($g.cm^{-2}$) was 16.5% and 19.5% lower in neck of femur and trochanteric regions respectively. But the deficit compared to the population age-matched mean was less than that seen in the spine (mean Z-score -0.6SD's for neck of femur and trochanteric regions in AM group). This suggests that Wolff's law applies equally in amenorrhoiec and eumenorrhoeic athletes but that running is an insufficient stimulus to prevent bone mineral loss associated with hypo-oestrogenism. Data from animal models of bone turnover (Rubin & Lanyon 1984) and weight-lifting athletes and gymnasts (Granhed *et al.*, 1987, Conroy *et al.*, 1993, Robinson *et al.*, 1995) also suggest that low impact, repetitive stress is less effective at inducing bone adaptation than high impact, low frequency stimuli.

The results presented here differ from most previous studies on femoral BMD in amenorrhoeic athletes. Amenorrhoeic dancers and gymnasts have been noted to have normal or high femoral BMD (Young *et al.*, 1994, Robinson *et al.*, 1995) which may reflect the different type of training. Previous studies on runners have primarily used dual photon absorptiometry (DPA) which is less sensitive at the proximal femur than

DXA which may account for some of the discrepancy (Drinkwater *et al.*, 1990, Myerson *et al.*, 1992). Snead *et al.*, (1992) showed no difference in BMD of femoral neck, trochanteric region or Wards triangle BMD between a group of 10 oligo/amenorrhoeic runners and eumenorrhoeic runners or sedentary controls. But this small group were defined as having 0 - 9 menses per year and were therefore more closely allied to the present study's OL group than AM group. Robinson *et al.*, (1995) did show a relationship between menstrual history and femoral BMD in runners although his group was too small to assess statistically. He found a 17% difference between those with prolonged amenorrhoea compared to those with regular cycles. Reduced BMD has also been noted in the femoral shaft by Drinkwater *et al.*, (1990) but in this original paper there was no difference in femoral neck BMD. A recently published paper from the same laboratory has however shown reduced bone density in all areas of the femur measured, thus supporting the present study (Rencken *et al.*, 1996). The strength of the present study lies in the relatively large number of amenorrhoeic athletes, separated from oligomenorrhoeic subjects, and the use of DXA, a more sensitive method of BMD measurement than Dual photon absorptiometry which was used in some of the earlier studies.

In common with Drinkwater *et al.*, (1990) we were unable to demonstrate any relationship between the duration of amenorrhoea and BMD. It has been suggested that during athletic amenorrhoea, the greatest rate of loss of bone mineral occurs during the first few years (Cann *et al.*, 1985). Our cohort of amenorrhoeic women had a mean of 7.75 years of amenorrhoea and it is likely that this obscured any relationship between BMD and the duration of amenorrhoea. More important in our study appeared to be the duration of eumenorrhoea, particularly in the trochanteric region and the lumbar spine. This suggets that the longer the skeleton is exposed to normal sex hormone levels at this age, the greater the ability to accrue bone mineral.

2) The beneficial effects of running on BMD

In common with other studies on the effects of weight-bearing exercise on BMD in regularly menstruating women, this study has shown that long-distance running is associated with high BMD at weight-bearing sites. In this group of eumenorrhoeic women, BMD of the proximal femur was not only 1 SD above the European age-matched mean, it was also just as high compared to the Hologic reference range for peak bone mass in American women. If bone mineral content of these women follow expected patterns of change they will have higher than average BMD at the menopause and will have a reduced lifetime risk of hip fracture (Melton *et al.*, 1988, Black *et al.*, 1992, Cummings *et al.*, 1993, Kröger *et al.*, 1995). As previously discussed, there is little benefit of running on BMD of the lumbar spine.

3) The effect of morphological characteristics on BMD

Some authors have suggested that lower BMD in amenorrhoeic athletes is due to their lower body weight (Myerson *et al.*, 1992) but in the present study the relationship with menstrual status remained very strong even after adjustment for these variables. Height, weight, and body mass index were significantly associated with BMD, predominantly in the lumbar spine although when menstrual group was accounted for, only height continued to contribute significantly to the variance in BMD. Within group analysis of these parameters however, showed that in the amenorrhoeic group, height and weight were independent predictors of BMD whereas this was not demonstrated in the other groups. These results are similar to those of Drinkwater (Drinkwater *et al.*, 1990, Rencken *et al.*, 1996) who has also shown that BMD is correlated with body weight and that this relationship is stronger in those athletes with the greatest menstrual dysfunction.

It is possible that in the presence of normal levels of sex hormones and large amounts of mechanical stress, other variables have only small additive influences on BMD

which we have been unable to demonstrate in this small sample. However, when sex hormone levels are low, as in athletic amenorrhoea, other factors may acquire more influence. It seems likely that mechanical stress to the skeleton is greater in taller, heavier women and this relationship is uncovered in the amenorrhoeic group.

4) Eating disorders and bone density

The prevalence of eating disorders was much greater in the amenorrhoeic group and a high anorexia score was associated with low body weight, body mass index and percentage body fat. Total EAT, Factor 1, Factor 3 and BITE scores were all correlated with BMD, predominantly at the trochanteric region but were not predictive of bone density when menstrual status was accounted for. These results support the evidence for a high incidence of eating disorders in amenorrhoeic runners. Gadpaille *et al.*, (1987) found that nearly two thirds of amenorrhoeic runners had evidence of an eating disorder compared to none of the cyclic runners in his study. Higher EAT scores and subscales of the score have also been found in amenorrhoeic runners compared to eumenorrhoeic runners (Myerson *et al.*, 1991) and prolonged amenorrhoea has been strongly associated with dieting in dancers. Brooks-Gunn *et al.* (1987) found that a half of amenorrhoeic dancers reported anorexia nervosa as opposed to only 13% of dancers with normal periods. The amenorrhoeic dancers weighed less and were leaner ($P<0.05$) than their eumenorrhoeic peers and had higher scores on the EAT26 scale ($P<0.01$). Although some workers have found no differences between the incidence of either overt anorexia and bulimia or altered eating behaviour or attitudes in amenorrhoeic athletes (Myburgh *et al.*, 1992; Snead *et al.*, 1992), the majority of workers have shown a trend towards some form of dysfunctional eating or weight control in those with menstrual abnormalities (Walberg & Johnston, 1991; Wilmore *et al.*, 1992) but no direct relationship with bone density.

There have been no other reports of the use of the BITE questionnaire in athletes. This questionnaire probes for the presence of bulimic symptoms in particular and it is interesting to note that the oligomenorrhoeic subjects had significantly high results compared to the eumenorrhoeic runners. Bulimia is often associated with normal weight, body fat and body mass index in addition to menstrual irregularity and it is possible that the oligomenorrhoeic athletes represent a distinct subgroup of long-distance runners.

Use of eating habits questionnaires such as the EAT26 and BITE may be helpful in identifying those athletes most at risk of developing abnormal eating habits and menstrual irregularity although they are not good independent predictors of bone density.

5) Dietary calcium intake and bone density

In addition to low caloric intake, athletes tend to avoid foods high in fat (often dairy products), and other studies have shown correlations between BMD and dietary calcium in runners (Wolman *et al.*, 1992). Although thirty per cent of our cohort consumed less than the British recommended daily intake of calcium (600mg/day) we found no differences in BMD at any region between those consuming either less than 600mg/day or more than 1000mg/day compared to the remainder. Nor was there any evidence of a linear relationship. It is possible that more detailed analysis of calcium intake, for instance by daily weighing of food or 7-day dietary records, may have revealed a closer relationship.

6) Conclusion

This study has confirmed the relationship between menstrual irregularity and low bone density in athletes, not only in the spine but also in the femur. Although weight and height contribute to some of the variance, this effect is far outweighed by the

enormous impact of the hypo-oestrogenic state. The study also highlights the interplay between low body weight, body mass index, eating disorders and amenorrhoea in this group of athletes.

Cross-sectional studies can be criticised for not eliminating selection bias. It is possible that those women who develop menstrual dysfunction have a constitutional tendency towards lower BMD, although there is evidence against this explanation (Slemenda *et al.*, 1994). This question cannot be fully answered until longitudinal studies follow adolescent athletes for many years. Furthermore, it is unknown whether amenorrhoeic athletes continue to have low BMD up to and after the menopause. There are suggestions that there may be a gain in BMD when menses return or when training is reduced (Lindberg *et al.*, 1987, Drinkwater *et al.*, 1986). However, it seems unlikely that these runners will ever reach their maximum potential peak bone mass. Until this question is resolved, athletic amenorrhoea should not be considered benign.

CHAPTER 6

REGULAR EXERCISE AND BONE MINERAL DENSITY IN THE DECADE BEFORE THE MENOPAUSE

Abstract

Objectives

To investigate whether regular vigorous physical exercise is associated with high levels of bone density in women in the decade before the menopause and whether this effect is less in women who start training after the attainment of peak bone mass.

To see if a previous history of menstrual irregularity has long-lasting effects on bone density despite exercise.

Methods

Bone density was measured by dual x-ray absorptiometry in 50 premenopausal women aged 40 years and over who each week exercised for at least three hours or ran 15 miles (24km). Twenty-seven began exercising after the age of 30 years (late-starters, LS); the remainder had been active since leaving school (all-timers, AT). Thirteen women had prior menstrual irregularity.

Results

There were no significant differences between the AT and LS groups in bone density of the lumbar spine and two sites in the proximal femur; but both groups showed high bone density at all three sites compared to the European reference range ($p<0.025$-0.005). Those who remained continuously eumenorrhoeic had bone density values 0.4 - 1.05 standard deviations above the mean for their age. Those with prior menstrual irregularity showed smaller benefits in the femur and none in the spine. Late-starters showed particularly high values in the femoral neck which were identical to all-timer athletes.

Conclusions

Bone density at common fracture sites was substantially greater than expected in women undertaking long-term exercise in the oestrogenized state whether exercise started in the 'teens or the thirties. Exercise may reduce the lifetime risk of fractures by preserving adequate bone mass into the later postmenopausal period.

Introduction

An ageing population and an increasingly sedentary lifestyle have contributed to the rising incidence of osteoporotic fractures in post-menopausal women (Cooper *et al.*, 1991). Because treatment of osteoporosis is often instigated only after fractures have occurred, methods of prevention are likely to be of greater benefit. A reduction in fracture rate of the proximal femur has been associated with increased levels of physical activity and muscle strength in the elderly (Cooper *et al.*, 1988). This may be due to fewer falls (Wickham *et al.*, 1989) but is also likely to be related to higher bone mineral density (BMD) in those individuals with greater levels of activity (Pocock *et al.*, 1989).

Although studies on older athletic women have shown that higher BMD is linked with greater physical activity (Jacobsen *et al.*, 1984, Talmage *et al.*, 1986, Jónsson *et al.*, 1992) little information exists on the long-term effects of regular exercise. Nor is it known what exercise prescription is required to achieve maximum bone mass. It is unclear whether exercise should be undertaken before the age when bone mass peaks or whether similar levels can be achieved if exercise is started after this age. High intensity exercise starting at an early age is associated with secondary amenorrhoea

and low oestrogen levels which may result in reduced bone density, especially of the lumbar spine (Marcus *et al.*, 1985, Drinkwater *et al.*, 1990, Wolman *et al.*, 1990).

This study assesses the long-term effects of regular exercise on bone density of premenopausal women forty years and over. It also examines the importance of the length of time during which the subject has exercised and the effects of previous episodes of menstrual irregularity.

Methods

Fifty premenopausal women, aged 40 years or over, were recruited after responding to advertisements in running and athletic journals. Subjects were from the general population of South-East England according to the criteria in Chapter 4. Twenty-three women had trained consistently since leaving school (all-timers, AT). The remaining 27 were sedentary after leaving school but commmenced training after the age of thirty years and for a minimum of three years prior to the study (late-starters, LS). Thirteen gave a history of menstrual irregularity lasting more than two years (non-EU). The others had had normal menstruation (EU) and all were eumenorrhoeic at the time of the study. These categories allowed further subdivision into four groups: 1) AT/EU, n=15; 2) AT/non-EU, n=8; 3) LS/EU, n=22; 4) LS/non-EU, n=5.

Questionnaires gave menstrual, obstetric and training histories. A general medical examination confirmed fitness to undertake the tests and informed consent was obtained.

Body Composition, Physiological Characteristics and Dietary Calcium Intake

As previously described in Chapter 4 (Methods) , the following measurements were made:

i) weight and height

ii) body mass index (BMI; weight/height^2)

iii) percentage body fat using the four-site skinfold thickness method and from total body potassium (TBK).

iv) fat free mass (FFM) in kg estimated from TBK.

v) grip strength of the dominant and non-dominant hands

vi) isokinetic back strength

vii) dietary calcium intake

Bone mineral density

Bone Mineral Density (BMD) in g.cm^{-2} of the lumbar vertebral bodies (L2-L4) and the left hip (neck of femur, FN, and trochanteric region, FT) was measured in each subject by dual energy x-ray absorptiometry (DXA), as described in Chapter 4. Scan analysis was performed by technicians who were blinded to the menstrual or activity status of the athlete. The BMD value of each region of interest was compared to the Hologic reference population at peak bone mass (T-score) and to the age-matched European population data (Z-score) as previously described.

T-scores and Z-scores were expressed as standard deviations (e.g. mean + 1SD = +1).

Statistical methods

The statistical package used was Microsoft Excel 5.0. Between group differences were assessed using ANOVA followed by Students 't' test. An independent 't' test compared the mean Z-scores and T-scores with the reference population. Simple correlation assessed relationships between physiological and morphological parameters. Linear regression with BMD g.cm^{-2} or Z-score as the dependent variable assessed relationships between BMD and the other variables. Multiple stepwise regression was then used to determine the most significant variables. Data values are

given as Mean (Standard Deviation, SD). Statistical significance is accepted at p<0 05 throughout.

RESULTS

1) Training Details

42 subjects were middle or long-distance runners, 3 were ultra-distance walkers and 5 competed in other events such as triathlon, hockey or javelin. Current and past sports are shown in Tables 1 and 2. Thirty (60%) competed at National or International veteran championship level and 20 (40%) competed at Club or County level. The groups were similar in number of miles run, hours spent training and number of sessions per week (Table 3). Weight-training was undertaken by 5 (33.3%) in group1, none in group 2, 8 (36%) in group 3 and 3 (60%) in group 4. There were no differences in BMD between those who had weight-trained and those who had not.

Late-starter athletes had exercised for a mean of 9.0 years (range 5 - 20 years) and were as successful at international level as the all-timer athletes (30%, 35% medals respectively). The older the woman in the LS group, the more years they were likely to have been training (correlation coefficient r=0.54, P<0.01).

2) Body composition and physiological parameters

Differences between the all-timer and late-starter athletes and between eumenorrhoeic and non-eumenorrhoeic athletes

The AT women were significantly taller (p<0.03) and had greater fat free mass in kg (p<0.05). When FFM was expressed as percentage of total body mass the difference between the two groups was less significant (p=0.067). The AT women also had a lower body fat content when assessed by skinfold thickness measurements (p<0.03)

but not when assessed by the TBK method (p=0.14). There were no other differences in strength measurements or body composition and maximal aerobic capacity (Table 4). There were no differences in training details, strength or physiological characteristics when grouped according to menstrual group (EU vs. Non-EU).

Strength and fitness parameters

The number of hours trained per week (HPW) correlated well with number of miles run (MPW) ($p<0.001$) and VO_2max (<0.05). VO_2max was also correlated with MPW and %body fat ($p<0.01$, $p<0.001$ respectively). There was no relationship between grip strength and aerobic fitness.

Dominant hand grip strength was correlated with most measures of back strength ($p<0.01$ - $p<0.05$) and non-dominant grip strength was highly correlated with all measures of back strength ($p<0.001$ - $p<0.01$). Grip strength was dependent on body weight ($p<0.05$).

3) Obstetric and gynaecological history

10 women had commenced hormone replacement therapy without a break in menses (2 in group 1, 1 in group 2, 6 in group 3 and 1 in group 4). One subject (group 1) had had a hysterectomy and bilateral oophorectomy with subsequent HRT administration. There were no significant differences in BMD of any region measured between this subgroup of eleven subjects and the whole sample. The EU women averaged 32.4 years of regular menses and the non-EU women had an average of 20.7 years of regular menses (ANOVA; $F= 61.8$, $p<0.0001$). Oligoamenorrhoea had occurred for 12.1 (6.5) and 5.2 (1.8) years in groups 2 and 4 respectively. The AT athletes had had fewer pregnancies than the LS group (mean 1.4 vs. 2.0, $p< 0.04$).

4) Dietary Calcium Intake

There were no differences across the groups in calcium intake. Only 6 subjects currently took less than the recommended daily allowance of $600mg.day^{-1}$ and 48%

consumed over 1000mg.day^{-1}. Although it was possible to assess past intake, this data was not felt to be sufficiently reliable to analyse. There were no linear correlations between BMD g.cm^{-2} or Z-scores and calcium intake. Nor was there any difference in BMD between those currently consuming >1000mg.day^{-1} and those taking less.

5) Bone Mineral Density

i) Results expressed as g.cm^{-2}

BMD in g.cm^{-2} are shown in Table 5.

Comparison by athletic activity and menstrual status

There were no significant differences between the femoral densities for EU and non-EU groups. However, BMD was lower in the lumbar spine in the non-EU group (P=0.045). BMD was similar in the AT and LS groups at all regions.

Regression Analysis

Linear regression of BMD (g.cm^{-2}) versus age showed no variation at any site. However when athletic status was accounted for, older age in the AT group was related to decreasing BMD in the lumbar spine and in the LS group was related to increasing L2-L4 BMD (P<0.05).

Neck of femur

There were no significant demographic, menstrual, or physiological variables associated with BMD of the FN.

Trochanteric region

Trochanteric BMD was positively associated with height and grip strength in the dominant hand. The greater the number of pregnancies, the lower the tendency for BMD (F=4.0, p=0.051). It was also negatively associated with the number of hours of training undertaken. However, this result was skewed by two individuals who trained

for 17.5 and 19 hours per week and in whom BMD was very low. When these results were excluded, this relationship was not apparent in the remainder of the cohort.

Lumbar Spine

Very similar results were obtained for L2-L4 as for FT except that the relationship between number of pregnancies and BMD was slightly stronger ($F=5.24$, $p=0.03$). Again the apparent relationship between hours trained and low bone density was skewed by two individuals. Lumbar BMD was also negatively associated with VO_2max.

Multiple regression

Menstrual group was used as the first independent variable followed by the parameters above. All the above noted relationships remained significant. When weight was accounted for the relationship between grip strength and lumbar BMD disappeared but was just outside significance for the trochanteric region ($p = 0.06$).

ii) BMD compared to the age-matched European population (Z-scores)

BMD Z-scores are shown in Table 6

The BMD of each subject was compared to the mean bone density found in the European reference population of that age. EU women (groups 1 and 3) had significantly high Z-scores in almost all regions. They were also significantly high in group 2 (AT/non-EU) in the neck of femur.

Comparison by athletic activity and menstrual status

BMD Z-scores were also analysed by athletic activity (AT vs. LS) and menstrual group (EU vs. non-EU). ANOVA showed no significant difference in BMD at any region between AT and LS. In addition, the mean Z-scores for the AT and LS groups were significantly above the age-matched mean (Graph 1). BMD was lower in non-EU athletes when compared to the EU women but this only reached significance in the lumbar spine ($P<0.05$), (Graph 2).

Linear regression (Appendix 2, table 2)

Neck of Femur

Again there were no apparent relationships between any of the variables measured and Z-scores.

Trochanteric region

As noted for $g.cm^{-2}$, FT Z-score was associated positively with height ($p<0.02$) and dominant hand grip strength ($p=0.054$). There was a similar negative association with number of pregnancies and hours trained per week.

Lumbar Spine

The same associations were found as for BMD $g.cm^{-2}$. Graphs 3 and 4 show the relationship between BMD Z-score and the number of hours trained per week and VO_2max respectively.

Multiple regression (Appendix 2, tables 3 and 4)

When menstrual status was accounted for in multiple regression, greater height was associated with higher Z-scores in the trochanteric region and the lumbar spine (both $p<0.05$). L2-L4 Z-score remained negatively associated with the number of pregnancies and VO_2max. The only additional finding was that spinal BMD became positively correlated with back strength (peak torque in extension at $60^{o}.sec^{-1}$, $p<0.05$, Appendix 2, table 4).

iii) BMD compared to the peak bone mass of the American population (T scores)

T-scores are shown in Table 7.

BMD of each subject was compared to the peak bone mass of that region found in the American reference population. EU women had positive T-scores in all regions but this was only significant in group 1 in the trochanteric region ($P<0.01$). Non-EU

women had negative T-scores except in the trochanteric region. The T-score in group 2 was low in the lumbar spine ($P<0.05$).

6) Biochemical Parameters

There were no differences between groups in any of the biochemical or hormonal parameters measured. In 4 women, oestradiol was below the detectable range of 70 $pmol.l^{-1}$ (1 in group 1, 1 in group 2, 2 in group 3). FSH was not above the normal range in these women and in view of regular menses, they were considered premenopausal. There was no correlation between oestradiol levels and BMD $g.cm^{-2}$. FSH was higher than the normal range in 7 women (2 in group 1, 1 in group 2, 3 in group 3, 1 in group 4). In all these women, oestradiol was within the normal range and 6 were on HRT. All had regular menses.

Table 1

Current Sporting Activity of Premenopausal Veteran Athletes

Sport	Definition	Number of athletes
Triathlon	Swim/cycle/run	3
Long-distance running	> 1500m - marathon	36
Middle-distance ruuning	800 - 1500m	4
Sprint	100/200/400m/hurdles	2
Race-walking	>1500m - marathon	3
Hockey		1
Throwing	Javelin/shot-putt/discus	1
Total		**50**

Table 2

Previous Sporting Activity of All-Timer Premenopausal Veteran Athletes

Sport	Definition	Number of athletes
Long-distance running	> 1500m - marathon	8
Middle-distance ruuning	800 - 1500m	5
Race-walking	>1500m - marathon	1
Ball games	Hockey/squash/badminton	6
Throwing	Javelin/shot-putt/discus	1
Cycling		1
Jumping	Long-jump/high jump	1
Total		**23**

Table 3

Training Characteristics of Premenopausal Veteran Athletes

Mean (Standard Deviation)

	Group 1 (AT/EU) n = 15	**Group 2 (AT/non-EU) n = 8**	**Group 3 (LS/EU) n = 22**	**Group 4 (LS/non-EU) n = 5**
Age (years)	45.5 (4.9)	42.5 (2.6)	46.0 (4.2)	43.2 (4.1)
Miles run per week	27.0 (17.5)	38.7 (13.7)	31.3 (15.8)	31.2 (8.2)
Hours trained per week	6.9 (4.3)	8.8 (5.0)	7.0 (2.4)	5.8 (1.5)
Number of training session per week	5.7 (2.4)	7.9 (3.8)	6.6 (1.7)	6.0 (1.7)

AT = all-timer, LS = late-starter, EU = always eumenorrhoeic, non-EU = at least 3 years prior menstrual

Table 4

Body Composition of Premenopausal Veteran Athletes
Mean (Standard Deviation)

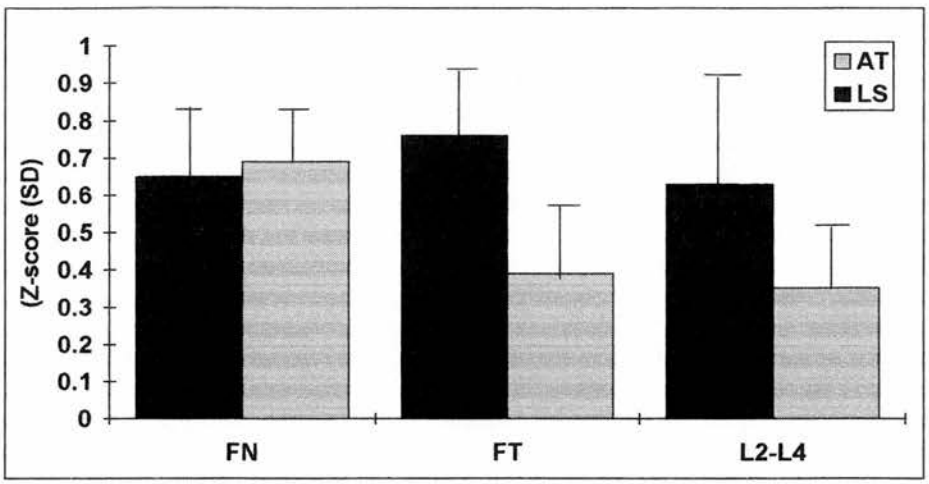
	Group 1 (AT/EU) n = 15	Group 2 (AT/non-EU) n = 8	Group 3 (LS/EU) n = 22	Group 4 (LS/non-EU) n = 5
Height (cm)	165.9 (4.8)**	163.5 (4.5)**	161.7 (4.2)	164.8 (4.5)
Weight (kg)	59.5 (4.9)	55.4 (5.5)	57.2 (4.2)	58.3 (6.0)
BMI ($kg.m^{-2}$)	21.6 (1.6)	20.7 (1.5)	21.9 (1.4)	21.4 (1.5)
Fat Free Mass (kg)	46.8 (3.2)*	45.1 (5.5)*	43.9 (3.9)	43.1 (4.9)
Fat Free Mass (%)	78.9 (3.9)	81.4 (6.0)	73.5 (8.5)	74.0 (5.9)
Body fat (%)	25.6 (2.0)**	23.7 (4.0)**	26.9 (5.0)	29.3 (3.5)
Grip strength				
Dominant	37.0 (5.8)	33.3 (3.1)	34.3 (3.8)	32.0 (5.1)
Non-dominant	33.9 (6.5)	31.6 (2.9)	30.9 (4.3)	30.1 (4.9)
(Kgf)				
VO_2max ($ml.kg^{-1}.min^{-1}$)	46.0 (6.8)	50.9 (7.4)	45.2 (8.3)	43.0 (11.2)

AT = all-timer, LS = late-starter, EU = always eumenorrhoeic, non-EU = at least 3 years prior menstrual irregularity.

Body fat (%) measured by skinfold thickness method

Students 't' test for AT(groups 1&2) vs. LS (groups 3&4): * $p<0.05$, ** $p<0.03$

Comparison of Bone Mineral Density Z-scores Between All-timer and Late-starter Athletes



Graph 1

Bone mineral density of 'all-timer' (AT) athletes and those who had started training after the age of 30 years ('late-starter', LS) athletes. Bone density shown as mean Z-score (+/-SE) for each region of interest. No significant differences found between the two groups at any region measured.

FN: femoral neck
FT: trochanteric region
L2-L4: lumbar spine

Table 5

Bone mineral density ($g.cm^{-2}$) of Premenopausal Veteran Athletes

Mean (Standard Deviation)

Group	n	Neck of Femur	Trochanteric Region	L2-L4
1 (AT/EU)	15	0.900 (0.13)	0.805 (0.10)	1.162 (0.17)
2 (AT/non-EU)	8	0.880 (0.05)	0.741 (0.07)	1.025 (0.05)*
3 (LS/EU)	22	0.901 (0.08)	0.753 (0.10)	1.088 (0.09)
4 (LS/non-EU)	5	0.863 (0.09)	0.723 (0.09)	1.051 (0.15)*

AT = all-timer, LS = late-starter, EU = always eumenorrhoeic, non-EU = at least 2 years prior menstrual irregularity.

* $p<0.05$ for EU vs. non-EU groups combined

Table 6

Comparison of BMD of Premenopausal Veteran Athletes with Age-matched European Data (Z-score)

Mean (Standard Deviation)

Group	n	Neck of Femur	Trochanteric Region	L2-L4
1 (AT/EU)	15	+0.71 (1.17)*	+0.97 (0.99)***	+1.05 (1.50)**
2 (AT/non-EU)	8	+0.52 (0.43)**	+0.35 (0.74)	-0.17 (0.53)
3 (LS/EU)	22	+0.77 (0.81)****	+0.45 (1.02)	+0.42 (0.83)*
4 (LS/non-EU)	5	+0.35 (0.84)	+0.15 (1.00)	+0.04 (1.40)

Z-score = SD's above or below the age-matched mean of the European population reference data

AT = all-timer, LS = late-starter, EU = always eumenorrhoeic, non-EU = at least 2 years prior menstrual irregularity.

't'-test of Z-score versus European data, P values: * <0.05, ** <0.02, *** <0.01, **** <0.001

Table 7

Comparison of BMD of Premenopausal Veteran Athletes with Peak Bone Mass of American Reference Population (T-score)

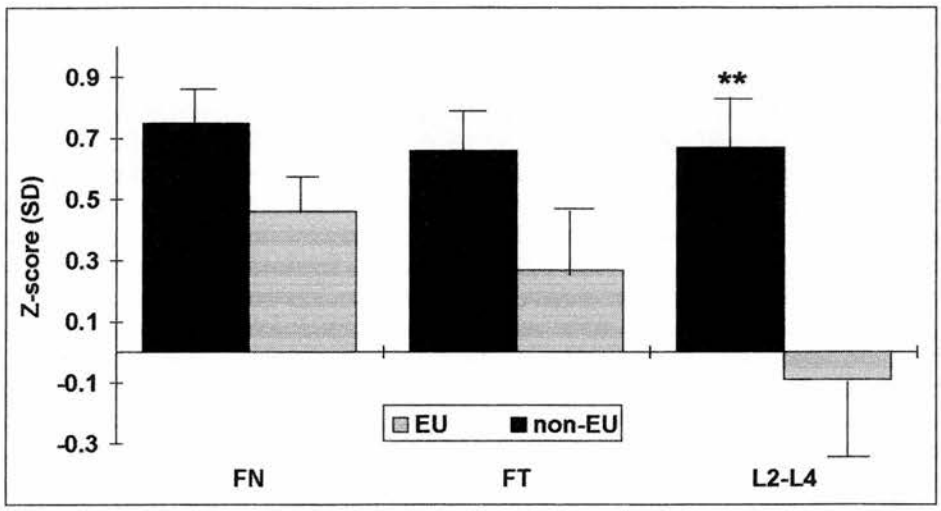
Mean (Standard Deviation)

Group	n	Neck of Femur	Trochanteric Region	L2-L4
1 (AT/EU)	15	+0.05 (1.31)	+0.92 (1.14)**	+0.75 (1.57)
2 (AT/non-EU)	8	+0.14 (0.45)	+0.21 (0.73)	-0.49 (0.48)*
3 (LS/EU)	22	+0.06 (0.82)	+0.35 (1.08)	+0.08 (0.83)
4 (LS/non-EU)	5	+0.31 (0.93)	+0.01 (0.96)	-0.25 (1.36)

T-score = SD's above or below the mean BMD of the Hologic reference population at 22 years for the neck of femur and 30 years for the trochanteric region and lumbar spine. AT = all-timer, LS = late-starter, EU = always eumenorrhoeic, non-EU = at least 2 years prior menstrual irregularity.

't' test for comparison of T-score versus mean of reference population, P values: * <0.05, ** <0.01

Comparison of BMD Z-scores between EU and non-EU Athletes

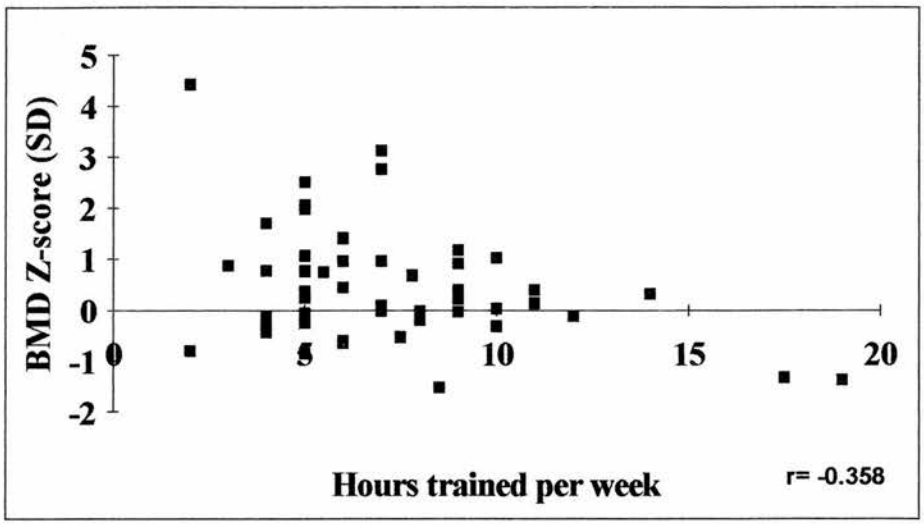


Graph 2

Bone mineral density of athletes who had had continuous eumenorrhoea (EU) compared to BMD of those who had a history of irregular menstruation (non-EU). Bone density shown as mean Z-score (+/-SE) for each region of interest. Although BMD is lower in all areas measured, this only reaches significance in the lumbar spine (**$p<0.05$)

FN: femoral neck
FT: trochanteric region
L2-L4: lumbar spine

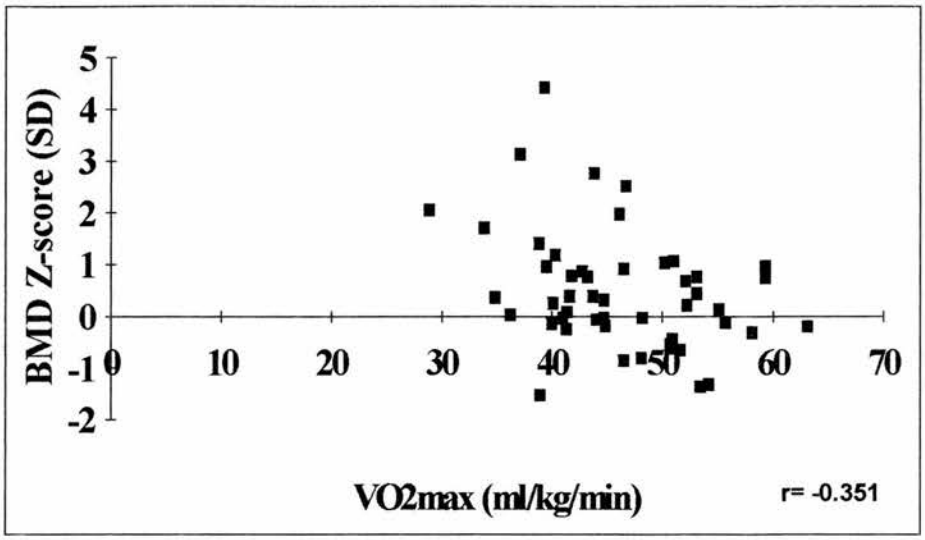
BMD Z-score of premenopausal veteran athletes versus number of hours trained per week



Graph 3

The age-adjusted bone mineral density (Z-score) was compared to the number of hours trained per week. The trend was for lower BMD in those who trained for the greatest number of hours per week. However this result was skewed by two individuals who trained for 17.5 and 19 hours per week and who had very low BMD. When these results were excluded the relationship was no longer apparent.

BMD Z-score of premenopausal veteran athletes versus maximal aerobic capacity (VO_2max)



Graph 4

The age-adjusted bone mineral density (Z-score) was correlated with the maximal aerobic capacity (VO2max). The trend was for lower BMD in those who had the highest aerobic capacity.

DISCUSSION

1) The effect on bone density of a lifetime of physical activity

Although this study was unable to show that bone density increases with exercise (as only a longitudinal study can), it has demonstrated that women with a lifetime history of regular aerobic exercise have much higher bone density than expected when compared to the European population. This was true for the lumbar spine as well as the weight-bearing proximal femur which perhaps reflects the slightly more diverse training in these older women than in the younger elite distance runners. Indeed, it was possible to demonstrate a correlation between back extensor strength and bone density of the lumbar spine. This has previously been shown in rowers by Wolman *et al.* (1990), in young women by Henderson *et al.*, (1995) and in older men by Bevier *et al.*, (1989).

Two other studies have looked at a similar group of women. Jacobson *et al.*, (1984) evaluated BMD in athletic women aged 40 - 55 years and found higher distal radius and lumbar BMD compared to their control population. This group of athletic women were predominantly tennis players and included both late-starter and all-timer athletes. The same group of workers also showed that the age-related loss in bone mineral was abolished in women who exercised regularly 3 times a week for 9 months of the year and who had done so for the last 5 years (Talmage *et al.*, 1986). There are no available equivalent studies for femoral bone density and no studies which have differentiated between late-starter and all-timer athletes.

Not only was bone density higher than age-matched European women, it was also higher than the Hologic reference mean for peak bone density in the trochanteric region. In these women who are approaching the menopause it is highly significant to find bone density which is approximately 1SD above the age-matched mean in all regions and nearly 1SD above peak bone mass in the femur. It has been shown by

several authors that high peak bone mass or high BMD at the menopause is associated with a reduced risk of osteoporotic fracture in the spine and the femur (Melton *et al.*, 1988, Black *et al.*, 1992, Kroger *et al.*, 1995). Cummings *et al.*, (1993) estimated from a group of 8000 postmenopausal women that those in the 90th percentile of femoral neck bone density had an 8% lifetime risk of fracture compared to a 25% risk for those in the 10th percentile. It seems likely therefore that the athletic women in this study will be at reduced risk of osteoporotic fracture.

2) The effect on bone density of regular exercise commenced after the age of peak bone mass

It is perhaps unsurprising that those women with a lifetime history of regular sport had high bone density. This could be predicted from all the studies on younger athletes which have shown high BMD in those undertaking both aerobic and high impact exercise. However, it has not been shown previously that women who were sedentary until after the age of peak bone mass (30 years) can have as high BMD. All of the subjects in this study were previously sedentary and none of them had had heavy manual jobs so it seems unlikely that this is due to selection bias although this cannot be ruled out. Indeed, cross-sectional population studies in women show that those who do not exercise have the lowest BMD (Zylstra *et al.*, 1989, Zhang *et al.*, 1992, Krall & Dawson-Hughes 1994). It seems more likely that significant beneficial effects were accrued by starting to exercise. This could occur by two means. Firstly that the age-related loss in bone mineral is reduced by regular activity as suggested by Jacobson *et al.*, (1984), Smith *et al.*, (1989) and Talmage *et al.*, (1986). Secondly, that bone mineral content is actually increased by the onset of exercise as shown in studies by Margulies *et al.*, 1986, Friedlander *et al.*, 1995 and Nichols *et al.*, 1994. This study has also shown that the longer the LS athlete had been training, the greater the lumbar BMD, suggesting ongoing beneficial effects on BMD.

3) Is there a limit to the volume of exercise which is beneficial?

Without doubt moderate amounts of exercise have beneficial effects on BMD. But data in this study suggest that very high levels of exercise may be detrimental to some regions of the skeleton. Lumbar spine and trochanteric BMD was found to be surprisingly low in two athletes who trained for very long periods each week (17.5 and 19 hours) although there was no correlation between number of miles run per week and BMD at any site. However, the lowest lumbar BMD was seen in those with the highest aerobic capacity measured by VO_2max. This is at variance with other studies that have demonstrated a positive correlation between lumbar BMD and VO_2max (Bevier *et al.*, 1989, Pocock *et al.*, 1989). However, one study in postmenopausal athletes has shown that high intensity exercise may be paradoxically associated with low BMD. Beat *et al.*, (1989) found lower than expected lumbar BMD in 50 - 60 year old women exercising for more than 300 minutes per week although in only two of these women was BMD extremely low. There may therefore be a non-linear relationship between BMD and exercise with the lowest BMD occurring at the two extremes of exercise. The reason for the negative correlation between high aerobic capacity and low lumbar BMD is unclear. It may be related to lower body fat levels and hence lower circulating oestrogen levels or other changes in morphology that have not been shown in this study. The relationship requires further evaluation.

4) The long-term effects of prior menstrual irregularity

Many studies have now demonstrated that young athletes with amenorrhoea have lower than expected BMD in the spine and other non-weight bearing sites (Cann *et al.*, Drinkwater *et al.*, 1984, 1990, Marcus *et al.*, 1985, Wolman *et al.*, 1990). Short-term follow-up studies have demonstrated some improvement in BMD following resumption of menses but at present there is no long-term follow-up.

In this study we hoped to assess the impact of a history of menstrual irregularity on BMD in older athletes who were now eumenorrhoeic. Duration of menstrual irregularity varied between 2 and 22 years with a mean of 12.1 years in the AT athletes and 5.2 years in the LS women. Bone density was lower in the non-EU women at all sites but this only reached significance in the lumbar spine ($p<0.05$). In addition there was no direct relationship between either the number of years of oligo/amenorrhoea or the number of years of eumenorrhoea and BMD (although the latter approached significance in the LS, $p = 0.08$).

Of importance was the finding that the Z-score was significantly high in the AT/non-EU group in the neck of femur and at no sites were Z-scores low. This suggests that, although there are long-term effects of prolonged menstrual dysfunction, exercise may help to offset these detrimental effects if menstruation returns. These findings need to be supported by longitudinal studies on amenorrhoeic athletes, a process that is currently underway.

5) Body composition and strength as determinants of bone mineral density

Several studies have demonstrated that height, weight and body mass index are significant independent determinants of BMD in pre and postmenopausal women (Pocock *et al.*, 1989, Lanham *et al.*, 1990, Nordin *et al.*, 1992, Rico *et al.*, 1993, Henderson *et al.*, 1995). In this study neither weight nor BMI were predictors of BMD but height was associated with trochanteric and lumbar BMD both before and after adjustment for age and menstrual status. It is unclear why weight was not an important determinant of BMD in this group of women.

In common with others, this study demonstrates a relationship between strength and BMD (Bevier *et al.*, 1989, Sinaki *et al.*, 1989, Pocock *et al.*, 1989, Kritz-Silverstein *et al.*, 1994, Henderson *et al.*, 1995). Back extensor strength was associated with lumbar BMD, and dominant hand grip strength predicted trochanteric and lumbar BMD although not when menstrual status or weight had been accounted for. It is likely that grip strength reflects aspects of total physical activity since back strength and grip strength were highly correlated. It could be a useful additional measure of strength and physical fitness in the general population when risk factors for low BMD are being assessed.

Conclusions

This study has demonstrated that bone mass is high in those that undertake regular physical activity. This may result in a reduction in osteoporotic fractures in later life. In addition, women who commence training after the age of 30 years are able to achieve similar bone density levels to those who have trained since school age. The greater number of years they train, the greater the effect on BMD. Some athletes have prolonged amenorrhoea due to their athletic activity and this results in reduced BMD. Although running is able to offset the loss in BMD in the femur, long-term effects of menstrual irregularity are seen in the lumbar spine. For the premenopausal woman,

provided she remains eumenorrhoeic, it may never be too late for her bones to benefit from exercise. If the differential in BMD at the femur between these athletes and controls is maintained in the future, their lifetime risk of fracture will be almost halved.

CHAPTER 7

BONE DENSITY IN POSTMENOPAUSAL ATHLETES

Abstract

Objectives To investigate the effects of vigorous physical activity on bone mineral density (BMD) in two groups of postmenopausal women. One group had exercised throughout their lives and the second group had started exercising after the age of 35 years.

Methods Bone mineral density of hip and lumbar spine was measured in 24 postmenopausal women (age range 48 - 68 years) who all trained for at least 3 hours per week or ran at least 15 miles per week. 10 had trained throughout their life (All-timers, AT) and 14 had started training after the age of 35 years (Late-starters, LS).

Results Linear regression of BMD ($g.cm^{-2}$) with age or years since menopause showed a decline in the trochanteric region $p<0.002$) and in the lumbar spine (L2-L4, $p<0.005$) but not in the neck of femur. There was no significant difference in BMD at any site measured between the AT and LS groups. Nor was BMD in either group different from the European reference range matched for years since menopause (YSM). BMD was significantly lower than the Hologic reference range for peak bone mass.

Linear regression revealed BMD (adjusted for YSM) of neck of femur and trochanteric region to be associated with weight, body mass index (BMI) and percentage body fat ($p<0.05$) There were no such associations with spinal BMD. Low body mass, percentage fat mass and BMI were associated with high aerobic capacity (VO_2max).

Conclusions In postmenopausal women, low BMD of the femur is associated with low weight and low body fat. High intensity training results in low body weight and

percentage body fat and may therefore reduce beneficial effects of exercise on BMD. Spinal BMD is not increased by running and is less influenced by body habitus.

Introduction

Regular physical activity has been associated with increased bone mineral density (BMD) in postmenopausal women (Jacobson *et al.*, 1984, Talmage *et al.*, 1986 Jónsson *et al.*, 1992, Zhang *et al.*, 1992, Krall *et al.*, 1993). Exercise training programmes have also been shown to increase BMD in post-menopausal women (Dalsky *et al.*, 1988). In addition to improving BMD, regular exercise increases muscle strength and reduces the frequency of falls (Wickham *et al.*, 1989, Province *et al.*, 1995, Wolf *et al.*, 1996). It is likely that these combined effects contribute to the lower rate of hip fracture seen in those with greater levels of physical activity (Cooper *et al.*, 1989). Methods of treatment of established osteoporosis can be unrewarding and interest is starting to focus on the possibility of exercise prescription for its prevention.

Although most exercise programmes in postmenopausal women have included either aerobic-type exercise (e.g. walking) (Dalsky *et al.*, 1988, Grove & Londeree, 1992, Martin & Notelovitz, 1993) or muscle-strengthening exercises (Chow *et al.*, 1987, Rikli & M^{c}Manis, 1990), there is very little information on either the long-term effects of such exercise or whether more intensive training would be more effective. However, two reports in veteran athletes have suggested that excessive exercise may have detrimental effects on BMD in the post-menopausal years (Nelson *et al.*, 1988, Michel *et al.*, 1989).

The aim of this study was to determine the effects of a lifetime of regular physical activity on BMD in postmenopausal women. We also wanted to determine whether it was possible to have significant effects on BMD by taking up regular exercise after the age of 35 years.

Methods

Twenty-four postmenopausal women were recruited after responding to advertisements in running and athletic magazines according to the criteria in Chapter 4. Women who were taking hormone replacement therapy (HRT) were not included. Subjects were divided according to whether they had trained consistently since school age (all-timers, AT) or whether they had commenced training after the age of 35 years (late-starters, LS).

Questionnaires gave menstrual, obstetric and training histories. A general medical examination confirmed fitness to undertake the tests and informed consent was obtained. Postmenopausal status was confirmed on serum measurements of follicle stimulating hormone and oestradiol.

Body Composition, Physiological Characteristics and Dietary Calcium Intake

As previously described in Chapter 4, the following measurements were made:

i) weight and height

ii) body mass index (BMI; weight/height^2)

iii) percentage body fat using the four-site skinfold thickness method and from total body potassium (TBK).

iv) fat free mass (FFM) in kg estimated from TBK.

v) grip strength of the dominant and non-dominant hands

vi) isokinetic back strength

vii) dietary calcium intake

Bone mineral density

Bone Mineral Density (BMD) in $g.cm^{-2}$ of the lumbar vertebral bodies (L2-L4) and the left hip (neck of femur and trochanteric region) was measured in each subject by dual energy x-ray absorptiometry (DXA), as described in Chapter 4. Scan analysis was performed by technicians who were blinded to the activity status of the athlete. The BMD in $g.cm^{-2}$ of each region of interest was compared to the Hologic reference population at peak bone mass (T-score) and to the European population data matched for years since the menopause (Z-score) as previously described.

T-scores and Z-scores were expressed as standard deviations (e.g. mean + 1SD = +1).

Statistical Analysis

Between group differences were assessed using a two-tailed Students 't' test. Linear regression was used to assess associations between measured parameters with BMD Z-score as the dependent variable where appropriate. An independent 't' test was used to compare the Z-scores and T-scores with the reference population. Significance was accepted throughout at the $p<0.05$ level.

RESULTS

1) Training Details

Ten women had trained consistently since leaving school (all-timers, AT). The remaining 14 women were sedentary after leaving school but commenced training after the age of 35 years (late-starters, LS). The all-timer athletes had undertaken a wide variety of sports previously (Table 1) but all were currently long-distance walkers or middle and long-distance runners. The late-starter athletes were all middle and long-distance runners and until the age of at least 35 years had undertaken less than 60 minutes per week of exercise (as defined by the American College of Sports Medicine, 1990 as the minimum requirement for the maintenance of cardiovascular fitness).

Twelve women competed at international and world class veteran championships and 10 had won medals in their age group in the last three years (5 in group AT, 5 in group LS). The other women competed at either National or club level (Table 1). All-timer athletes had exercised for a mean of 36.6 years (range 31 - 43) and LS athletes had trained for a mean of 7.5 years (range 4 - 15).

The AT athletes ran more miles per week than the LS athletes ($p<0.05$) but otherwise training characterisitics were similar between the two groups (Table 2).

2) Body composition and physiological parameters

All-timer athletes were taller than the LS athletes ($p<0.02$) but otherwise physical, characteristics were similar in the two groups (Table 2). Although the AT athletes were stronger in both hands and had greater aerobic fitness, these differences were not significant. The AT athletes had greater flexor and extensor trunk strength than the LS athletes but this was only significant for flexor strength at the faster torque speed (180^{o} sec^{-1}).

There was no correlation between the number of miles run per week or the number of hours trained per week and weight, percentage body fat or body mass index. However, maximal aerobic capacity (VO_2max) was highly correlated with percentage body fat ($p<0.002$, Graph 1) and body mass index ($p<0.005$). Low weight was also associated with high VO_2max ($p=0.059$). VO_2max declined with age ($p<0.03$).

3) Obstetric and gynaecological history

There were no differences between the two groups for age at menarche, approximate number of years of regular menstruation or number of years postmenopausal. Three AT women and 7 LS women had used the oral contraceptive pill (mean years of usage, 1.05, 0.82 respectively). Three AT women and 5 LS women had used HRT

(mean years of usage, 2.6, 2.9 respectively). There were no differences between the two groups in the number of pregnancies or the number of months spent breastfeeding.

4) Dietary calcium intake

There were no differences in calcium intake between the two groups. Only 2 athletes consumed less than 600mg.day^{-1} and 14 athletes consumed more than 1000mg.day^{-1}

5) Bone Mineral Density

There were no significant differences in BMD at any region between the AT and LS groups (Table 3) and they were therefore combined for further analysis by linear regression.

Linear Regression

i) BMD g.cm^{-2}

Bone density declined with age and 'years since menopause' (YSM) in the trochanteric region and the lumbar spine ($p<0.05$) but there was no such trend in the neck of femur.

ii) BMD compared to the European population (Z-score)

BMD of AT and LS athletes was not significantly different from the European reference range for postmenopausal women when matched for YSM. (Table 2, Graph 2).

Table 4 shows the associations between physical characteristics and Z-scores

Neck of femur: BMD Z-scores were positively associated with weight ($p<0.05$), BMI ($p<0.005$) and % body fat ($p<0.02$).

Trochanteric region: BMD Z-scores were positively associated with weight ($p<0.005$, Graph 3), BMI ($p<0.02$) and % body fat ($p<0.05$) High BMD in the trochanteric region was associated with high fat free mass ($p<0.05$). In this region,

strong associations were found between dominant and non-dominant grip strength and Z-scores, (p=0.001, p=0.0005 respectively, Graph 4)

Lumbar spine: There were no associations between BMD Z-score and any of the variables measured.

There was no correlation between BMD (any region) and miles run per week, hours spent training per week or number of sessions per week. Back strength was not fouund to be a predictor of bone density in any area. Additionally there was no difference in BMD between those who were most successful in competition and those who competed at a lower level or between those training for more than 5 hours per week or more than 40 miles per week and those training less hard.

iii) BMD compared to the peak bone mass of the American population (T-score)

As expected, BMD was significantly lower than the average peak bone mass of each region in American women, according to the reference range utilised by Hologic (Table 3).

6) Biochemical Parameters

There were no differences between the two groups in biochemical parameters measured. Measurement of levels of oestrogen and FSH confirmed postmenopausal state. In one athlete aged 63 years, levels of FSH were extremely low suggesting hypothalamic suppression or insufficiency in addition to hypogonadism. Later investigations on this subject found normal levels of other pituitary hormones and she was felt to have hypothalamic suppression of FSH secondary to her training.

Table 1

Group	Previous sport	Sport now	Level now
All-Timers (AT)			
1	100/200/400	LDR	World (6th 10k)
2	sprint hurdles	LDR	National
3	200/400/800	LD/MDR	World (M)
4	discus/pent	golf/keep fit/athletics	World (MR)
5	Racquet sports	LDR	World (M)
6	Racquet sports	LDR/golf	Club
7	MDR	LDR	World (M+R)
8	800/1 mile	800m/LDR	National
9	Rowing	LD walking	International (MR)
10	100 - 800m	LDR	National
Late-Starters (LS)			
1		LDR	World (M)
2		LDR	International
3		LDR	Club
4		LDR	National (M)
5		MDR/XC	National
6		Tri (Run/Swim/Shoot)	National (M)
7		LDR	World (MR)
8		LDR	Club
9		LDR	World (M)
10		LDR	World (M)
11		LDR	World (M)
12		LDR	National
13		LDR	Club
14		LDR/Golf	Inter-island

Key

LDR Long-distance running
MDR Middle-distance running
M Medal winner at National or World Championships
R World record holder for that age group

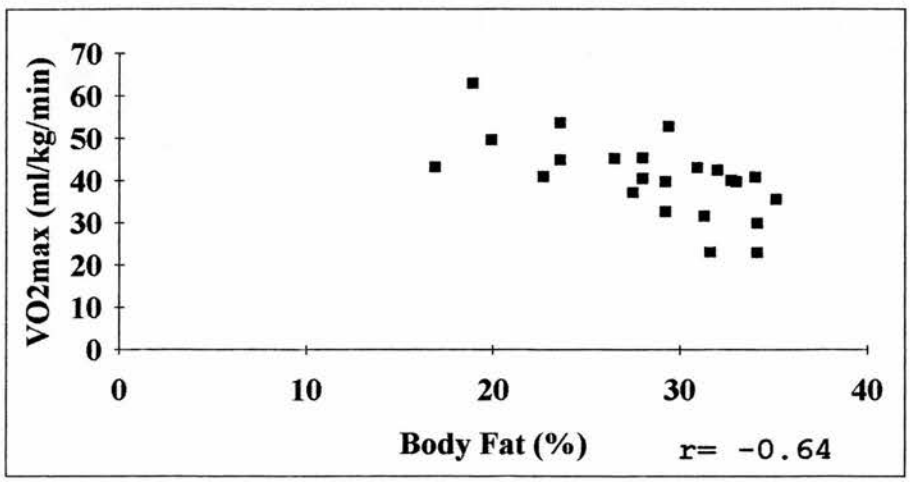
Table 2 **Subject Characteristics**

Mean (SD)

	Group AT (n=10)	**Group LS** (n=14)
Age (years)	54.6 (4.4)	56.5 (6.2)
Years since menopause	6.4 (5.0)	6.8 (5.5)
Years on HRT	1.0 (1.9)	0.8 (1.7)
Height (cm)	167.1 (6.7)	160.8 (4.6) *
Weight (kg)	60.9 (8.0)	57.7 (7.0)
Body Mass Index ($kg.m^{-2}$)	21.7 (1.6)	22.3 (2.3)
% Body Fat	28.2 (4.2)	28.5 (5.8)
Fat Free Mass (kg)	46.3 (7.3)	42.4 (5.2)
VO2max ($ml.kg.^{-1}min.^{-1}$)	43.1 (6.8)	39.4 (10.6)
Age started training		48.9 (6.0)
Miles Run per Week	36.1 (15.7)	25.0 (9.3)
Hours Training per Week	7.3 (2.6)	6.1 (2.3)

Students 't' test: *$p<0.02$

Correlation Between Maximal Aerobic Capacity (VO_2max) and Percentage Body Fat



Graph 1

This graph demonstrates the strong relationship between physical fitness, as measured by maximal aerobic capacity (VO_2max), and percentage body fat in postmenopausal athletes

$r = -0.64$, $p < 0.005$

Table 3 **BMD of Proximal Femur and Lumbar Spine.**

Mean (SD)

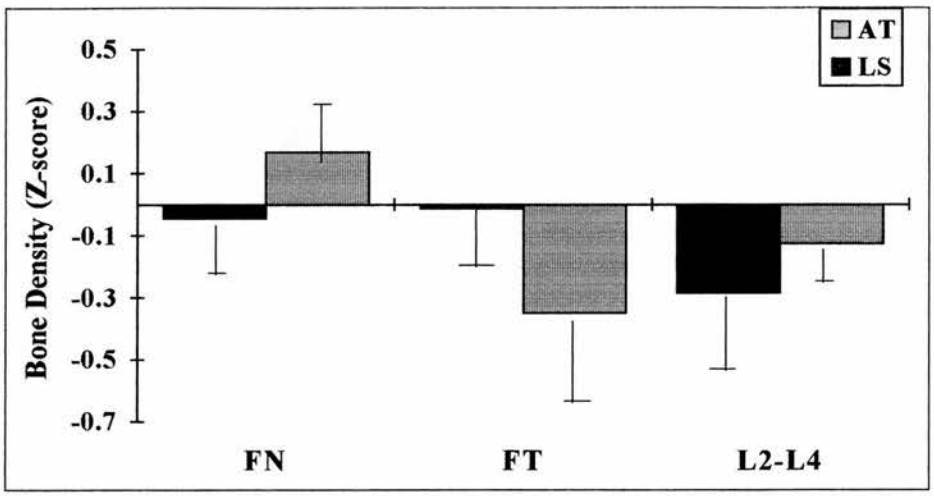
Group	Neck of Femur	Trochanteric Region	Lumbar Spine L2-L4
	G.cm^{-2}	**G.cm^{-2}**	**G.cm^{-2}**
AT	0.759 (0.06)	0.675 (0.06)	0.959 (0.11)
LS	0.783 (0.12)	0.632 (0.11)	0.910 (0.09)
	Z-score	**Z-score**	**Z-score**
AT	-0.044 (0.63)	-0.014 (0.53)	-0.285 (0.81)
LS	+0.168 (1.11)	-0.349 (1.01)	-0.127 (0.70)
	T-score	**T-score**	**T-score**
AT	-1.357 (1.25)****	-0.525 (0.66)*	-1.073 (0.96)***
LS	-1.115 (1.25)**	-0.996 (1.28)**	-1.535 (0.82)****

BMD values in g/cm2 and in comparison with European data (Z-score) and peak bone mass of American reference population (T-score). No differences between groups and neither group significantly different from matched European data. T-scores significantly lower than peak bone mass of American reference range:

t-test: * $p<0.02$, ** $p<0.01$, *** $p<0.002$, **** $p<0.001$

AT: All-timers
LS: Late-starters

Bone Mineral Density (Z-score) of Proximal Femur and Lumbar Spine

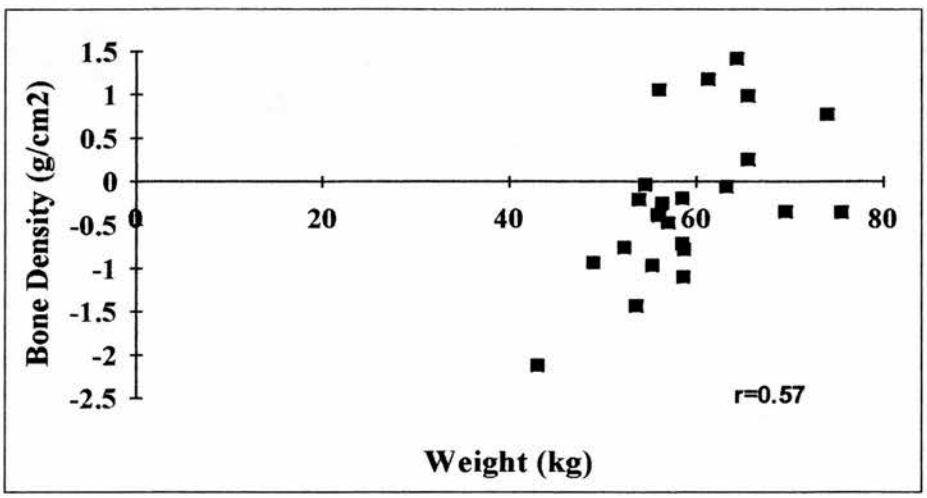


Graph 2

Mean (+/- SE) of bone mineral density compared to the European reference range matched for years since menopause (Z-score). No significant differences found betweeen the two athletic groups or compared to the European range.
AT: 'all-timer' athletes who had trained consistently since school age
LS: 'late-starter' athletes who had taken up athletics after the age of 35 years.

FN: femoral neck
FT: trochanteric region
L2-L4: lumbar spine

Bone Mineral Density (Z-score) of Trochanteric Region of Proximal Femur versus Body Weight

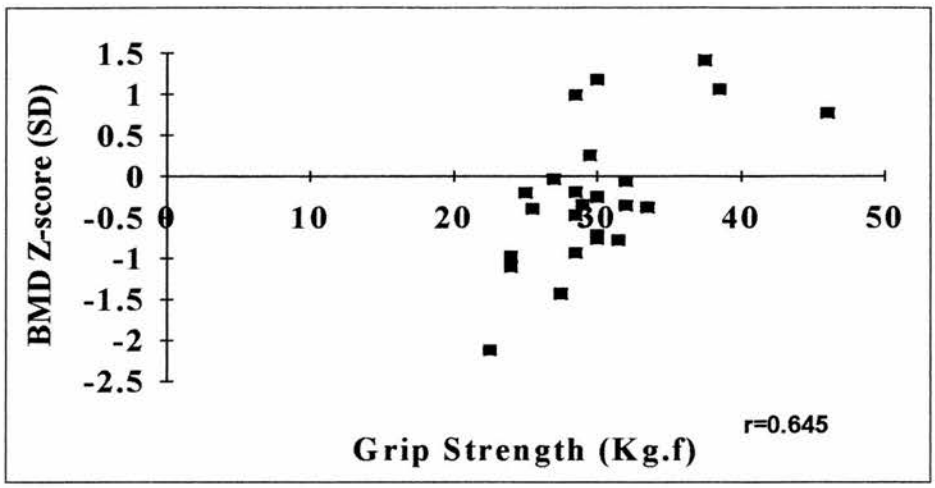


Graph 3

Bone mineral density (Z-score) of trochanteric region of proximal femur increases with increasing body weight in postmenopausal women whether 'all-timer' or 'late-starter' athletes.

$r = 0.57$, $p<0.01$

Bone Mineral Density (Z-score) of Trochanteric Region of Proximal Femur versus Grip Strength of Non-Dominant Hand



Graph 4

This demonstrates the positive linear relationship between grip strength and distant bony sites such as the trochanteric region ahown here. Bone density shown adjusted for years since the menopause (Z-score).

$r = 0.045$, $p<0.005$

Table 4 **Associations between BMD (Z-score) and Physical Characteristics**

Linear Regression Analysis

Linear regression with:	**Neck of Femur**		**Trochanteric Region**	
	F ratio	p value	F ratio	p value
Weight (kg)	5.398	0.035	10.316	0.003
BMI ($kg.m^{-2}$)	10.423	0.004	6.484	0.014
Body fat (%)	7.417	0.016	3.635	0.045
Fat free mass	0.235	0.671	4.187	0.032

Discussion

1) The effect of a lifetime of physical activity

In this small section of the overall study, ten women were included who had exercised continuously since school age (a minimum of 31 years exercise). The results from the previous two sections predict that these postmenopausal women should have had higher bone density than average throughout their adult years and into the menopause. The finding that their BMD was no greater than expected for their postmenopausal age was surprising and raises several possibilities.

Firstly, the level of exercise that they undertook during their younger years was insufficient to generate an increase in BMD. Most of these women trained for and competed in their chosen sport at National or International standard and although intensity of training was lower 30 years ago than it is now, it is unlikely it was insufficient to influence bone metabolism. None of the athletes had a history of prolonged menstrual irregularity.

Secondly, if BMD of this group was high at the menopause there must have been a rapid loss of bone mineral in the early postmenopausal years. Other studies (Smith *et al.*, 1989, Grove & Loneree, 1992, Martin & Notelovitz, 1993) have shown that exercise intervention in the postmenopausal years can slow the normal rate of bone mineral loss in sedentary women and it is therefore unlikely that this is the explanation. If BMD was low at the menopause in this group despite exercise it could be said that continued physical activity in the postmenopausal years has actually helped maintain BMD. These questions are unanswerable in the context of a small cross-sectional study but are pointers for areas to be pursued in long-term prospective studies of athletic women as they go through the menopause.

An explanation that will be explored further in this discussion is that intensive exercise in this group resulted in low body mass and fat mass and this has resulted in a BMD appropriate to that mass.

2) The effect of regular exercise commenced after the age of peak bone mass

This group of women were somewhat mixed as some had started exercise before the menopause and some after. This seemed to have no effect on their BMD which was very similar to that of the previous group. The cautions and explanations applied to the all-timer athletes are also applicable here.

There are two previous reports of small numbers of older men and women who trained intensively and who were found to have paradoxically low levels of BMD. Michel *et al.*, (1989) found that 5 of 28 women over 50 years, who were undertaking aerobic weight-bearing exercise for more than 5 hours per week had lower than expected lumbar spine bone density. Women exercising less than this showed a positive correlation between amount of exercise and BMD. In a 2 year prospective follow-up study to this, Michel *et al.*, (1990) confirmed that, in females over the age of fifty, extreme levels of exercise (greater than 300 minutes per week) were associated with low lumbar bone density. Nelson *et al.* (1988) compared 18 sedentary women with 15 endurance-trained postmenopausal women who ran a mean of 22.6 miles per week (mean age, 62.2 years). They found no difference in BMD of radius, proximal femur or lumbar spine between the sedentary women and those undertaking large amounts of exercise although. The results in this study support these previous findings and suggest that, in this age group, large quantities of aerobic exercise such as running are not associated with high bone density in either the femur or lumbar spine.

Slightly lower levels of repetitive aerobic exercise or other forms of activity may be more beneficial to skeletal metabolism. Studies by Jacobsen *et al.*, (1984) and

Talmage *et al.*, (1986) found that postmenopausal women who exercised for one hour three times weekly at the intensity of a game of tennis did have higher radial and spinal bone density than controls. And, as discussed previously, studies on younger athletes, both male and female, have shown that the greatest stimulus to bone formation appears to be weight-training. It is possible therefore that there is a non-linear relationship between amounts of aerobic exercise and bone density with low density occurring at the two extremes of exercise. It is important to evaluate this relationship further as many more postmenopausal women are now undertaking vigorous forms of exercise. Before exercise programmes can be endorsed for prevention of osteoporosis, it is necessary to determine just how much is enough.

3) Body composition as a determinant of bone mineral density

Studies on pre- and postmenopausal women have found that body weight and body mass index are independent determinants of bone mineral density (Pocock *et al.*, 1989, Lanham *et al.*, 1990, Nordin *et al.*, 1992, Henderson *et al.*, 1995, Johnell *et al.*, 1995, Orwoll *et al.*, 1996). Obese women have a lower incidence of osteoporotic fracture than women of normal habitus (Ribot *et al.*, 1994). There are two postulates for this. Firstly that skeletal stress is less in lighter women resulting in a lower stimulus to bone remodelling. Secondly, androgens are aromatised to oestrogens in peripheral fat and in postmenopausal women this may be an important source of oestrogens so that in very lean women, levels of circulating oestrogens are likely to be lower. It is likely that both are contributory.

These hypotheses may explain the paradoxically low bone density in this group of highly active women. As a group, they had a body mass index similar to women under the age of 30 years and certainly much lower than the mean for 50 - 64 year old British women (25.2 +/-0.24 SE)*. Some of the women had very low levels of body fat and the two subjects with the lowest percentage body fat also had two of the

lowest bone densities. In view of these observations, it could be said that in this group of women undertaking high levels of exercise, the muscular stresses of running helped maintain bone density despite low body mass and fat.

4) Strength and physical fitness as determinants of bone mineral density

Bone density of the spine and femur have been found to correlate with maximal aerobic capacity (VO_2max). In a study of 91 elderly non-athletic men and women, aged 61 - 84 years, spinal bone density was assessed by dual photon absorptiometry and VO_2max assessed by treadmill ergometry (Bevier *et al.*, 1989). Spinal BMD was correlated with VO_2max in the men but not in the women and in neither group was BMD of the radius correlated with VO_2max. Pocock et al (1989) found that BMD of the proximal femur correlated with physical fitness (VO_2max) in 73 healthy female volunteers aged 20 - 75 years.

The present study was unable to show a direct relationship between maximal oxygen uptake or volume of exercise (miles per week or number of hours spent training) and bone density, but there was a trend for those women with very high aerobic capacities to have the lowest percentage body fat, weight and body mass index. To a large extent this reflects oxygen uptake which is measured in units per kilogram of mass. Only the active muscles will have higher uptake during exercise and excess fat will reduce the unit measured. However, it may also suggest that the training required to maintain or improve aerobic capacity also results in low weight and body fat levels. Unfortunately we were not able to further elucidate particular kinds of training which most affected these factors. Evaluation of training intensity rather than volume may have been more helpful. Adjusting the VO_2max for age may also have helped evaluate this relationship further. Other studies have used submaximal fitness testing and a wider age range of subjects which may partly explain the discrepancy between this study and others.

Muscle strength has been shown to correlate with bone density in several studies. In the above study by Bevier *et al.*, grip and back strength were correlated with forearm and spinal BMD in the men and grip strength predicted forearm and spinal bone density in women. Pocock *et al.* (1989) also found that biceps and quadriceps muscle strength predicted bone mass in the forearm, femur and lumbar spine in female volunteers aged 20-75 years. In postmenopausal but not premenopausal women, grip strength wasa predictor of forearm BMD. Back extensor strength has also been found to correlate with bone density of the spine by Sinaki and Offord (1988) and Eickoff *et al.* (1993). Sinaki *et al.* (1993) found that osteoporotic women aged 40 - 85 years had lower back extensor strength than women with normal bone density. The results of the current study show a strong correlation between grip strength and BMD but only in the trochanteric region. In addition, back strength was not correlated with bone density at any site even after adjustment for body weight. Other studies have used non-athletic women and this, combined with small numbers may explain the discrepancy between these results and past studies.

In conclusion, this study differs from most other reports on exercise and bone density in postmenopausal women which have shown higher BMD in those that take most exercise. However, other studies have used either non-athletic women or women who train modestly. In this group of highly athletic women, low body weight and body fat may have resulted in bone density which is lower than expected given the amount of physical exercise they undertake. This supports similar findings of two other small studies on endurance-trained postmenopausal women. In view of these observations, further research is required to determine the type and volume of exercise required to preserve or increase bone mass in postmenopausal women.

CHAPTER 8

TREATMENT OF REDUCED BONE MINERAL DENSITY IN ATHLETIC AMENORRHOEA: A PILOT STUDY

Abstract

Objectives To determine the influence of hormone replacement therapy (HRT) and calcium supplementation on bone mineral density in athletes with irregular menstruation.

Design 18 month randomised prospective interventional study

Subjects 34 middle and long-distance runners, aged 18 - 35 years, with either oligomenorrhoea or amenorrhoea induced by intensive exercise, were randomised to three groups: A) to receive hormone replacement therapy (HRT) and 1000mg calcium per day (n=10), B) to receive 1000mg calcium per day (n=14), C) a control group who received no treatment (n=10).

Main outcome measures Comparison of pre and post-treatment bone mineral density (BMD) of proximal femur and lumbar spine within and between groups at 9 and 18 months. Comparison of BMD between those who regained menstruation and those who remained amenorrhoeic or oligomenorrhoeic.

Results Twenty-six athletes attended for at least one follow-up. Only 17 athletes returned for a second follow-up appointment. Numbers were insufficient for analysis at 18 months. Only results from the first follow-up are presented. There were no differences between pre and post treatment BMD in any group or between the groups. Results were then analysed according to whether menstruation returned (either naturally or secondary to HRT (EU)) or whether subjects remained amenorrhoeic (AM). During the first year BMD increased in the EU group in all areas and decreased in all regions in the AM group. The estimated differential change between the two groups over the first 12 months was 5.8%, ($P<0.02$) in L2-L4, 5.6% ($P<0.02$) in Wards area, 3.9% in the trochanter ($P<0.05$) and 2.4% in neck of femur (NS). Serum oestrogen levels rose from

275 pmol.l^{-1} to 589 pmol.l^{-1} in the EU group and fell from 110 pmol.l^{-1} to 96pmol.l^{-1} in the AM group.

Conclusions The increase in oestrogen levels associated with resumption of menstruation or treatment with HRT appears to be associated with an increase in BMD in amenorrhoeic athletes. In athletes who remained amenorrhoeic, BMD declined.

Power calculations On the basis of an 'intention to treat' analysis, a study designed to show, with 80% power and 5% significance, a benefit in lumbar spine BMD of HRT treatment would require about 1150 athletes with amenorrhoea or oligomenorrhoea. These large numbers are due to incomplete follow-up, withdrawal from treatment with HRT and return of menstruation in the non-HRT groups. These numbers might be reduced substantially by confining a definitive study to amenorrhoeic athletes, who appear less likely to regain menses.

Introduction

Menstrual dysfunction due to intensive exercise is now well recognised and is associated with a reduction in bone mineral density, particularly in the lumbar spine (Marcus *et al.*, 1985, Drinkwater *et al.*, 1990, Wolman *et al.*, 1990). Some studies have shown BMD in the vertebral bodies to be as as much as 25% below eumenorrhoeic peers and there are concerns that these athletes may be at increased risk of early osteoporosis and fracture. Reports on small numbers of amenorrhoeic athletes suggest that BMD of the vertebral bodies may increase when menstruation returns in response to a reduction in training volume and weight gain (Drrinkwater *et al.*, 1986, Lindberg *et al.*, 1987). Unfortunately there is no long-term data on BMD in these women and it remains unclear whether bone density continues to increase when menstruation returns or whether it stabilises at low levels. In view of the uncertainty, prevention of bone loss early in athletic amenorrhoea appears a sensible option although the means by which this could be achieved are not yet determined.

Many athletes are unwilling to reduce training or increase weight so drug intervention may have to be used. The anti-androgen cyproterone acetate in its contraceptive form (which includes large amounts of ethinyl oestradiol) has been shown to increase BMD in the lumbar spine (De Cree *et al.*, 1988) in small numbers of athletic women and Prior *et al.* (1991) in a brief report have shown a 2 to 3% increase in spinal bone density over a year using medroxyprogesterone. Additionally, in elite athletes who take the oral contraceptive pill, BMD is similar to that seen in eumenorrhoeic athletes (Wolman *et al.*, 1991). Some form of hormonal intervention may therefore be of benefit in either treating low bone density or reducing further bone loss. Not all athletes are likely to favour hormonal treatment and in these, calcium may have a part to play. Calcium supplementation has a small but significant effect on reducing bone loss in perimenopausal (Elders *et al.*, 1994) and postmenopausal women (Cumming, 1990). Dietary calcium intake has also been correlated with BMD of the lumbar spine in female athletes (Wolman *et al.*, 1992) and calcium and vitamin D3 supplementation may reduce the rate of fractures in elderly women (Chapuy *et al.*, 1992). The effect of calcium supplementation on BMD in female athletes has so far been shown to be of little benefit (Baer *et al.*, 1992).

Recently a survey of physicians involved in the primary care of female athletes revealed that 92% would use some form of hormone therapy and 87% would use calcium supplementation in athletic amenorrhoea despite the lack of scientific evidence to support their efficacy (Haberland *et al.*, 1995). In order to answer some of the questions regarding treatment of amenorrhoeic athletes, the effect of hormone replacement therapy and calcium on BMD in thirty-four runners with menstrual dysfunction was studied. The aim of this pilot study was to assess the feasibility of a full scale study in this relatively rare group of athletes.

Methods

34 middle and long-distance caucasian runners aged 18 - 35 years were recruited from advertisements in running magazines. Subjects were from the general population of England and Wales and fulfilled the criteria detailed in Chapter 4. Nine subjects had 4 - 9 menstrual cycles per year for at least the last eighteen months (oligomenorrhoea, OL). Twenty-five subjects had 0 - 3 cycles per year for at least the last eighteen months (amenorrhoea, AM).

Questionnaires gave menstrual, obstetric and training histories. A general medical examination confirmed fitness to undertake the tests and to receive treatment. Informed consent was obtained and approval was granted by the Ethical Committee of Northwick Park Hospital.

Body Composition, Physiological Characteristics and Dietary Calcium Intake

As previously described in Chapter 4 (Methods), the following measurements were made:

i) weight and height

ii) body mass index (BMI; weight/height^2)

iii) percentage body fat using the four-site skinfold thickness method

iv) dietary calcium intake

v) maximal aerobic capacity (VO_2max)

Bone mineral density

Bone Mineral Density (BMD) in g.cm^{-2} of the lumbar vertebral bodies (L2-L4) and the left hip (Wards Triangle, FW, neck of femur, FN, and trochanteric region, FT) was measured in each subject by dual energy x-ray absorptiometry (DXA), as described in Chapter 4. Scan analysis was performed by technicians who were blinded to the treatment status of the athlete. The DXA scans were reanalysed by the same technician and the same scan area was used on each occasion aided by Hologic software. Bone

density in $g.cm^{-2}$ was compared to the European reference range as described in Chapter 4. Z-scores were expressed as standard deviations above or below the mean.

Biochemical measurements

Serum levels of oestrogen and follicle stimulating hormone were measured at each visit. Serum prolactin, calcium and alkaline phosphatase levels and urinary β human chorionic gonadotrophin were measured to exclude other causes of secondary amenorrhoea or reduced BMD.

Treatment

Subjects were randomly assigned to one of three groups:

A) daily oral treatment with Trisequens™ tablets (oestriol 1mg and oestradiol 2mg for 12 days; oestriol 1mg, oestradiol 2mg and norethisterone acetate 1mg for 10 days; oestriol 0.5mg and oestradiol 1mg for 6 days) + 1000mg calcium carbonate (Sandocal™) (n=10)

B) 1000mg calcium carbonate (Sandocal™) orally per day (n=14);

C) Control group, no treatment (n=10).

Statistical analysis

Standard statistical tests were performed using the Microsoft™ Excel 5 statistical package. Pre and post treatment bone density ($g \cdot cm^{-2}$) was compared using a paired 't' test. The 9 month data were compared using a standard analysis of variance of the annualized changes between groups. In addition, the results were used to perform power calculations for a full study of HRT's effectiveness, as described by Armitage (1971). In these calculations the assumption was made that HRT users who withdrew from treatment would show the same range of bone density changes as the control group, the opposite applying to controls who naturally regained their menses.

RESULTS

25 athletes were amenorrhoeic (mean of 0 menses per year) and 9 athletes had oligomenorrhoea (mean of 5.7 menses per year). Groups were similar in age, height, weight, body mass index and training details (Table 1). Mean number of years of menstrual irregularity was 6.9 (group A), 7.2 (group B) and 6.7 (group C). Starting BMD was similar in all regions (Table 3). Mean BMD for the whole cohort was: -0.45, -0.30 and $^{-1}$.0 standard deviations below European age-matched reference data in the neck of femur, trochanteric region and lumbar spine respectively. Age-matched data were not available for Wards area. Daily calcium intake at the start of the study was 986mg (group A), 815mg (group B) and 1037mg (group C).

Follow-up

26 athletes returned for at least one repeat measurement at a mean interval of 9.3 months (group A), 11.2 months (group B) and 11.7 months (group C). Reasons for withdrawal are shown in Table 2. During this first treatment period, one subject in group A and 2 in group B stopped treatment because of side effects. Calcium commonly caused gastrointestinal upset. HRT caused breast tenderness, emotional lability and subjective weight gain (not independently verified).

Despite intensive efforts to maintain compliance, the drop-out rate was high and only a further 17 (50%) subjects, (6 in group A, 6 in group B and 5 in group C), returned for a second follow-up appointment. Of these 75% had continued on treatment. Reasons for withdrawal during the second stage are shown in Table 2. In total, it was known that 4 of the original 10 athletes on HRT and 2 of the 14 on calcium stopped therapy because of side effects.

Because of the limited follow-up at 18 months, only the results obtained at the first follow-up are presented. There were no differences between those that dropped out and those that continued the study in any of the baseline parameters measured except for

calcium intake. Estimated average calcium intake in the former group was 442mg.day^{-1} compared to 939day^{-1} in the latter group (p= 0.001) .

Resumption of menstruation

During the first treatment phase, 4 athletes in group B and 3 in group C became eumenorrhoeic. Only 1 of the 13 amenorrhoeic subjects not on HRT started to menstruate naturally whereas all 6 of the oligomenorrhoeic subjects commenced normal menstruation.

Bone Density Results

Paired 't' tests showed no difference between pre and post treatment BMD in any group. The change in BMD (in g·cm^{-2}) between the first two appointments were then extrapolated to a change per year to account for the different times in reattendance. The mean values for changes in BMD are shown in Table 3. ANOVA found no differences in post-treatment BMD between the groups.

The subjects were then categorised according to whether normal oestrogen status had been restored (either with HRT or natural menstruation). This gave a group of 14 athletes with 'normal' oestrogen status (EU) and 12 athletes that remained amenorrhoeic or oligomenorrhoeic (AM). Paired 't' test showed a significant increase in the BMD of the EU group in Wards triangle and the lumbar spine ($p<0.05$). In all regions measured, BMD increased in the EU group and fell in the AM group (Table 4). The annualised differential change between the two groups over the first 12 months was 5.8%, ($P<0.02$) in L2-L4, 5.6% ($P<0.02$) in Wards area, 3.9% in the trochanter ($P<0.05$) and 2.4% in neck of femur (NS) (Graph 1).

Serum oestrogen levels rose from 275 (+/-281) pmol.l^{-1} to 589 (+/-521) pmol.l^{-1} in the EU group and fell from 110 (+/-39) pmol.l^{-1} to 96 (+/-34) pmol.l^{-1} in the AM group.

Table 1 **Physical and Training Characteristics**

Mean (SD)

Group	Age (years)	Height (cm)	Weight (kg)	BMI ($kg \cdot m^{-2}$)	Miles per Week	VO2max ($ml \cdot kg^{-1} \cdot min^{-1}$)
A	28.4 (4.8)	163.4 (6.1)	53.6 (5.6)	19.5 (1.0)	45.0 (20.7)	57.0 (7.7)
B	28.7 (6.0)	165.0 (3.7)	51.1 (5.9)	18.8 (2.1)	55.0 (22.5)	56.0 (7.9)
C	25.0 (5.3)	162.7 (5.0)	51.5 (6.2)	19.5 (2.2)	53.1 (13.8)	61.8 (4.7)

Group A - oestriol, oestradiol and norethisterone acetate (Trisequens™)

- calcium carbonate 1000mg (Sandocal™) per day

Group B - calcium carbonate 1000mg (Sandocal™) per day

Group C -no treatment

Table 2 **Reasons for Withdrawal from Study**

	Withdrawal in First Treatment Phase		**Withdrawal in Second Treatment Phase**
Group		**Group**	
A	Non weight-bearing rest for bilateral femoral stress fractures	A	Chronic fatigue syndrome
A	Lost to follow-up	B	Lost to follow-up
A	Wanted to commence OCP	B	Chronic fatigue syndrome
B	Wanted to commence HRT	B	Chronic fatigue syndrome
B	Lost to follow-up	B	Wanted to commence OCP
B	Wanted to commence OCP	C	Wanted to commence OCP
B	Wanted to commence HRT	C	Wanted to commence OCP
C	Lost to follow-up	C	Lost to follow-up
		C	Training abroad

OCP - oral contraceptive pill, HRT - hormone replacement therapy.

Those athletes who wanted some form of hormone therapy and who were not in the appropriate group withdrew in order to commence treatment.

Tables 3a-d **Changes in Bone Mineral Density of Proximal Femur During First Treatment Phase**

Mean BMD ($g.cm^{-2}$)(SD)

Table 3a **Neck of Femur**

Group	Neck of Femur		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
A (n=7)	0.827 (0.10)	0.823 (0.10)	-0.63 (2.21)
B (n=10)	0.782 (0.06)	0.784 (0.05)	+1.33 (6.29)
C (n=9)	0.846 (0.10)	0.844 (0.12)	-0.03 (4.14)

Table 3b **Trochanteric Region**

Group	Trochanteric Region		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
A (n=7)	0.692 (0.13)	0.700 (0.12)	+1.71 (2.86)
B (n=10)	0.668 (0.11)	0.663 (0.09)	-0.33 (5.21)
C (n=9)	0.699 (0.09)	0.681 (0.10)	-1.89 (6.29)

Table 3d **Wards Triangle**

Group	Wards Triangle		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
A (n=7)	0.704 (0.15)	0.721 (0.13)	+3.55 (4.05)
B (n=10)	0.656 (0.09)	0.659 (0.08)	+1.37 (9.00)
C (n=9)	0.774 (0.12)	0.768 (0.13)	-0.83 (5.21)

Table 3c **Lumbar Spine (L2 - L4)**

Group	Lumbar Spine (L2-L4)		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
A (n=7)	0.941 (0.16)	0.974 (0.13)	+5.67 (9.47)
B (n=10)	0.965 (0.11)	0.959 (0.11)	-0.03 (5.01)
C (n=9)	0.929 (0.10)	0.922 (0.11)	-0.29 (4.62)

Bone mineral density shown in $g.cm^{-2}$ for before and after treatment at 9.3 months (group A), 11.2 months (group B) and 11.7 months (group C). To allow for different times of reattendance, individual changes in BMD were adjusted to give % change per year. Analysis by ANOVA found no differences between groups.

Group A: oestriol, oestradiol and norethisterone acetate as daily hormone replacement therapy (Trisequens™) + calcium carbonate 1000mg (Sandocal™) per day

Group B: calcium carbonate (Sandocal™) 1000mg per day

Group C: no treatment

Table 4a-d **Changes in Bone Mineral Density according to menstrual history during treatment phase**

Mean BMD ($g.cm^{-2}$)(SD)

Table 4a **Neck of Femur**

Group	Neck of Femur		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
EU (n=14)	0.821 (0.15)	0.830 (0.11)	+1.42 (4.35)
AM (n=12)	0.811 (0.05)	0.798 (0.04)	-0.94 (4.84)

Table 4b **Trochanteric Region**

Group	Trochanteric Region		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
EU (n=14)	0.697 (0.11)	0.705 (0.10)	+1.48 (2.85)
AM (n=12)	0.670 (0.10)	0.649 (0.10)	-2.42 (6.45)

Table 4c **Wards Triangle**

		Wards Triangle	
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
EU (n=14)	0.714 (0.16)	0.733 (0.15)*	+3.12 (6.34)
AM (n=12)	0.704 (0.07)	0.690 (0.07)	-1.88 (5.42)

Table 4d **Lumbar Spine**

Group	Lumbar Spine L2-L4		
	Pre-Treatment ($g \cdot cm^{-2}$)	**Post-Treatment ($g \cdot cm^{-2}$)**	**Change in BMD (% per year)**
EU (n=14)	0.964 (0.13)	0.991 (0.10)*	+4.11 (6.88)
AM (n=12)	0.925 (0.11)	0.902 (0.11)	$^{-1}$.73 (4.98)

Tables 4a-d:

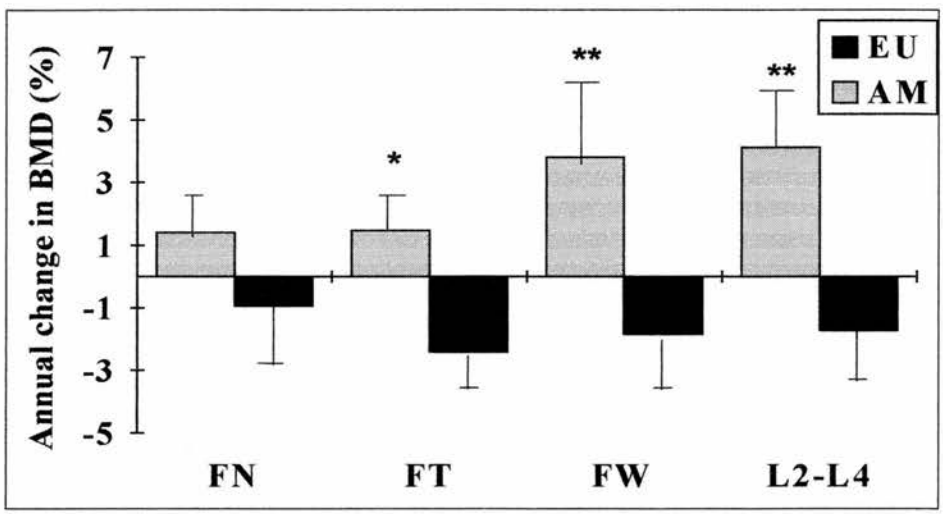
BMD in $g \cdot cm^{-2}$ for pre and post treatment at 9.7 months (group EU) and 12.2 months (group AM).

Group EU: all athletes that commenced menstruaton naturally and those on HRT

Group AM: all athletes that remained amenorrhoeic or oligomenorrhoeic

Paired 't' test for pre and post treatment BMD: *$P < 0.05$

Annual Change in Bone Density According to Menstrual History (%)



Graph 1

Athletes were grouped according to whether they resumed regular menstruation (EU, n=14) or whether they remained amenorrhoeic or oligomenorrhoeic (AM,n=12) during the first year of the study. Follow-up was at a mean of 9.7 months for the EU group and 12.2 months in the AM group. The change in BMD ($g.cm^{-2}$) was annualised to account for the different times of reattendance (bars indicate mean +/- standard error).

Those who regained normal menstrual cycles naturally were grouped with those who were on HRT (n=14). Four athletes in the calcium treatment group and 3 in the control group regained regular menstruation.

ANOVA for the difference between the two groups: *$p<0.05$,**$p<0.02$
FN = femoral neck, FT = trochanteric region
FW = Wards triangle

Power calculations for a full study of the effectiveness of HRT in amenorrhoeic athletes using LS BMD as the principal end-point.

(See also Appendix 4)

Although the difference between the changes in Lumbar Spine (LS) BMD in subjects regaining a normal oestrogen status exceeded 5% annually (Graph 1), because these data were recalculated from 9 months, it was assumed that, in an 18 - 24 month study, HRT would result in a 5% lumbar spine increase relative to baseline in the treated group and controls would not change. From Table 3c, the SD for a change in lumbar spine BMD is 6.37% of inital BMD value. If 30% of the control groups regain their menses and gain 5%, the mean increase in the controls is projected to be 1.5%. If, as within this pilot study, 40% of the HRT group withdrew from treatment, the mean increase is only 3.0%. The mean expected difference in an 'intention to treat' analysis is therefore only 1.5%. Therefore, for a power of 80% and significance of 5%, 280 are required to submit to follow-up densitometry in each group. However, in this pilot study, only 50% attended at 18 months so recruitment would have to be doubled to 560 in each group.

However, if the study was confined to athletes with amenorrhoea (<3 cycles per year), only 8% would be expected to regain menses. The mean expected difference in BMD would then be 2.6% and recruitment could be reduced to 188 in each group.

This power calculation was skewed by the outlying result of an annualised increase of 25.62% in lumbar spine BMD in one athlete. Exclusion of this result would decrease the standard deviation to 4.49 and recalculation results in a total number of athletes required to be 280 which could be further reduced to 46 if only amenorrhoeic athletes were used.

Discussion

Previous work on small numbers of amenorrhoeic athletes have suggested that menstruation may return if training levels are reduced and/or weight is gained. This resumption in menstruation has been associated with an increase in vertebral BMD of approximately 6.5% over 15 months (Drinkwater *et al.*, 1986, Lindberg *et al.*, 1987). Not all athletes are likely to accept either of these methods in order to resume menstruation and it has therefore been suggested that some form of hormone replacement therapy may be used to protect against bone mineral loss (Prior *et al.*, 1992) though until recently there was little research to support this. With this pilot study it was hoped to determine likely changes in BMD with either HRT or calcium treatment and from this to calculate the numbers of athletes required for a full-scale study.

In this study, in subjects treated with hormone replacement therapy, small but non-significant gains in BMD occurred at most regions measured. Additionally, in all regions, BMD fell by small amounts in the control group although it was only in the trochanteric region that BMD fell by more than 1% which is the in-vivo precision errror. The results were most convincing at the lumbar spine and Wards triangle where the difference between the two groups was 6.0% and 4.5%. Although these results are on small numbers and there is a large variance, they do suggest a trend towards at least a maintenance of bone mineral in those treated with HRT. Due to the small numbers, we were unable to determine whether changes in BMD were similar in the first and second treatment phases. Work on postmenopausal women supplemented with HRT suggests that increases in BMD are maximal in the first 12 months (Johnston & Melton, 1993). Whether a similar pattern occurs in younger women will require more prolonged studies than presented here.

The results in this study are more significant when the subjects are regrouped into those who remained amenorrhoeic during the first year and those who had some form of restoration of oestrogen levels either by resumption of normal menstruation or by HRT. Not only did BMD increase by between 1.4% and 4.1% over 10 months in the eumenorrhoeic group but BMD decreased by between 0.9% and 2.4% in the amenorrhoeic group. Again this difference was most marked in the lumbar spine and Wards area. Smaller effects are seen in the trochanteric region and little effect in the neck of femur. This may reflect different rates of bone turnover associated with higher levels of trabecular bone in the lumbar spine and parts of the proximal femur. These results are similar to those reported by Prior *et al.* (1994) and if BMD had continued to increase over a 15 month period, these results would have been similar to those previously published for athletes that regain natural menstruation.

Although there is little available research on treatment of bone loss in amenorrhoeic athletes, lesons can be learnt from the management of women with other forms of amenorrhoea. Haenggi *et al.,* (1994) treated 15 amenorrhoeic women, with proven low BMD, with ethinyloestradiol and desorgestrel over a 12 - 24 month period and also found significant gains in BMD in the lumbar spine and Wards Triangle, the maximum gain being 2.9%. These results are very similar to the 2.1% increase per year in the lumbar spine seen by Gulekli *et al.* (1994) who treated 85 women aged 17 - 40 years with amenorrhoea due to various causes. These authors used a number of different formulations of oestrogen replacement, all of which resulted in significant increases in BMD. Additionally, those women who received no hormone replacement but in whom weight gain occurred also had increased BMD. Although the women in the above two studies had a mixture of hypothalamic and ovarian amenorrhoea it is unlikely that this obscures the significance of their findings for those women with athletic amenorrhoea.

Anorexia nervosa is another disorder which is invariably associated with amenorrhoea and profound loss of bone mineral. Klibanski et al (1995) randomized 48 anorexic women to receive oestrogen and progestin replacement or no therapy. Over the follow-up period of 18 months, treated patients maintained BMD whilst untreated patients lost as much as 20% of their bone mineral.

It seems likely that treatment with some form of hormone replacement, whether by the resumption of natural menstruation, the oral contraceptive pill or HRT, results in at least small gains in BMD. Clearly though, loss of bone mineral can be prevented in amenorrhoeic women and for this reason, early intervention within the first six months should be advocated.

Power calculations for design of a full-scale study based on the findings of this pilot study suggest that for a comparison of HRT with no treatment, very large numbers of amenorrhoeic and oligomenorrhoeic athletes would be required (at least 1150). However these two groups of athletes may have different long-term menstrual function. All of the formerly oligomenorrhoeic athletes became eumenorrhoeic during the course of the study whereas almost all of the amenorrhoeic athletes remained without menses. Therefore, by confining recruitment to truly amenorrhoeic athletes, the differential change in BMD over an 18 - 24 month treatment period would be greatly increased and only 380 athletes, or fewer, would be required. Multi-centre trials on such numbers might be possible.

These results do not show any significant effect on BMD of calcium supplementation and in all athletes in this group who remained completely amenorrhoeic, BMD continued to fall. In two athletes the decrease in BMD was substantial (7% - 8% in the first year). A low dietary calcium intake has been associated with low BMD in female athletes. Wolman (1992) found cross-sectionally that for every 100 mg increase in daily

calcium intake, lumbar trabecular bone density rose by 3.9 $mg \cdot cm^{-3}$ (measured by quantitative computed tomography scanning) although he cautioned against assuming there was a cause and effect relationship. In a study of seven adolescent amenorrhoeic runners, supplementation with 1200mg calcium carbonate and 400IU vitamin per day for 12 months did not prevent bone loss in two of the athletes and increases in bone density were not as great as in the control group of eumenorrhoeic athletes (Baer et al, 1992). In postmenopausal studies, the change in BMD with calcium supplementation has been modest (in the order of 1%) (Cumming *et al.*, 1990) and it seems likely that if any beneficial effect is to be seen in athletes, very large numbers of subjects will be required.

Fifty per cent of the athletes withdrew from the study or were lost to follow-up. Many of the reasons given reflect the nature of the population studied. If follow-up could be improved, perhaps by more frequent contact or by providing greater benefit to the athlete e.g. by fuller physiological assessment, fewer numbers would be needed for a full study of this kind. Side effects were also a problem. In those that returned for follow-up, 35% had stopped treatment during the 18 month period. HRT caused breast tenderness and a subjective sensation of weight gain, particularly during the progestogen phase of the cycle. Additionally, several athletes noticed emotional lability that caused two to stop taking therapy. Side effects of breast tenderness and weight gain are obviously a particular problem for runners. Possibly a different formulation more suitable for younger women may reduce these side effects. Calcium also caused problems, predominantly gastrointestinal. Diarrhoea and abdominal cramps were sufficiently severe to prevent continuation of treatment in two subjects. If athletes are to accept medication, it has to be with minimal side-effects that do not impair performance or interfere with training.

A small study of this nature can only provide some indication of where future research may best be aimed. It is difficult to recruit large numbers of amenorrhoeic athletes that fulfill all the requirements of such a study and, as shown here, many athletes do not continue to have menstrual irregularity. The power calculations suggest that only a large multicentre trial would be likely to produce the answers that are so urgently needed.

CHAPTER 9

CONCLUSIONS

The prevention and management of osteoporosis is a challenge not just at the present but also for the future. If the forecasts of the increase in the rate of fracture in the UK are correct then huge sums of money will be expended on the treatment and rehabilitation of elderly patients with osteoporotic fractures. Not only will money be spent, but considerable morbidity and mortality will be suffered. But this need not occur if methods of prevention of osteoporosis can be improved. Screening of those at risk of osteoporosis can be undertaken but the correlation between osteoporosis and risk factors is not good and time and money may be spent on those who are at low risk of fracture. Primary preventative measures must at least play a part in reducing the burden of osteoporotic fractures on society. Indeed the recent Mediterranean Osteoporosis Study (MEDOS, Lyritis, 1996) found that the majority of hip fractures could be explained on the basis of the potentially reversible risk factors assessed in the study, one of which was low levels of physical activity.

There are many approaches which could be utilised to increase the peak bone mass of the population and to preserve bone mass into old age. Just one of these measures is to increase the average level of exercise in women of all ages. This study has contributed to the accumulating mass of evidence that eumenorrhoeic young women who regularly exercise have substantially higher bone density at important fracture sites compared to the average population. It has also shown that older women who undertake regular exercise in the decade before the menopause, have significantly higher bone mass than expected. The most surprising finding in this group of women is the extent to which bone density is preserved in those women who came into

exercise later in life. This is particularly encouraging to many women who start to exercise after family commitments have eased.

However very intensive exercise may result in oligomenorrhoea or amenorrhoea and evidence is accumulating that such menstrual disturbance has profound effects on bone mineralisation. Initially most studies demonstrated this effect in the spine but this study has shown quite clearly that the deficit in bone density between amenorrhoeic and eumenorrhoeic runners is similar whether measured at a weight-bearing or non weight-bearing site. Although site-specific exercise can offset these effects to some extent, it is clear that maximum potential peak bone mass in these athletes is unlikely to be achieved. But there has been a gap in our knowledge about the long-term outcome of bone density in such women. This study has gone someway to address this gap. The work on the older premenopausal athletes with a history of menstrual irregularity suggests that continued exercise may at least maintain or possibly increase bone density at certain sites provided oestrogen levels are normalised. In this group of women, who were predominantly runners, this resulted in non-significant differences in BMD in the femur between those who had been continuously eumenorrhoeic and those with prior menstrual irregularity. But long-term effects were seen in the lumbar spine which is less affected by weight-bearing exercise, and although bone density was not lower than average at this site, the skeletal benefits of exercise had been lost.

These findings place even more emphasis on the need for early recognition and active management of athletic amenorrhoea. There is still much ignorance within the sporting community about this disorder, not only amongst the support staff but also amongst the athletes. A strong treatment plan is important and as yet there is no concensus on the best management approach or the likely results of treatment. The treatment intervention study included in this thesis supports other work suggesting that any form of oestrogen replacement, whether natural or synthetic, results in a

small but significant increase in bone density. What remains unknown is the extent to which this recovery may be maintained. The problems highlighted in this pilot study will enable suitable multicentre longitudinal studies to be planned.

It appears too that high levels of aerobic exercise in the hypo-oestrogenic state associated with the menopause may be deleterious to the skeleton. The relationship between morphology and bone density is particularly strong after the menopause and, by lowering their weight and body fat, those women who exercise intensively may end up with paradoxically low bone density. It is likely that there is a level at which the amount of aerobic-type exercise is sufficient to stimulate bone remodelling but not sufficient to result in low body weight and fat. It is even more likely that the best form of exercise to promote in the elderly is a mix between aerobic exercise and weight-training. However it is clear that exercise prescription is not straightforward at any age and each person must be assessed individually in order to achieve the maximum potential benefit to the skeleton.

Finally, cross-sectional studies such as this can only give a snapshot of the overall picture and perhaps give ideas for further research. This has indeed been the case and a longitudinal study is underway which will endeavour to follow these active women through the menopause and beyond. This longitudinal study may help to determine why there is such a discrepancy in bone density between the premenopausal and postmenopausal women. It will also establish whether bone mineral can be preserved by continued exercise in the face of falling oestrogen levels. Even longer-term is the aim to repeat bone density measurements on the amenorrhoeic young athletes as they approach the menopause, a plan that will be harder to fulfill.

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APPENDIX 1

DATA AND FURTHER STATISTICAL ANALYSIS FOR ATHLETES AGED 16 - 35 YEARS

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Demographic Variables

Number of Athlete	Age (years)	Height (cm)	Weight (kg)	Body Mass Index ($kg.m^{-2}$)	Body Fat (%)
Amenorrhoeic Group					
1	27	165.0	52.0	19.10	17.6
2	28	161.8	55.0	20.96	21.1
3	20	166.0	52.5	19.05	19.7
4	30	169.0	53.9	18.87	21.0
5	18	166.5	48.7	17.57	12.6
6	35	165.0	50.0	15.15	15.2
7	28	159.0	50.6	20.02	21.5
8	21	166.5	44.5	16.05	11.3
9	30	163.0	48.0	18.07	17.9
10	32	156.0	51.5	21.17	24.7
11	26	167.0	52.0	18.65	22.7
12	34	166.0	54.0	19.60	20.2
13	27	165.0	47.0	17.26	14.8
14	29	171.0	57.6	19.70	21.3
15	27	164.0	37.6	13.98	7.1
16	34	165.0	52.0	19.08	15.9
17	25	160.0	52.7	20.59	21.7
18	22	172.0	59.2	20.01	15.6
19	32	162.0	52.3	19.93	20.6
20	28	156.0	46.0	18.90	13.8
21	31	162.0	52.5	20.00	18.0
22	30	175.0	63.0	20.57	20.6
23	19	164.0	48.7	18.11	16.8
24	20	163.0	49.0	18.44	19.3
Oligomenorrhoeic Group					
1	27	169.0	57.0	19.96	25.7
2	18	163.0	63.5	23.90	17.8
3	28	163.0	48.5	18.25	18.7
4	21	160.0	56.5	22.07	21.1
5	35	166.5	57.5	20.74	25.1
6	18	169.0	50.5	17.68	22.8
7	29	156.0	50.2	20.63	21.7
8	19	161.0	45.3	17.48	15.4
9	34	166.0	56.0	20.32	23.2

Eumenorrhoeic Young Athletes

Demographic Variables

Number of Athlete	Age (years)	Height (cm)	Weight (kg)	Body Mass Index ($kg.m^{-2}$)	Body Fat (%)
1	28	171.0	58.5	20.01	20.8
2	16	159.0	50.0	19.78	18.9
3	31	169.0	57.6	20.17	19.8
4	23	163.0	52.4	19.72	16.9
5	18	165.0	52.4	19.25	21.3
6	32	169.0	60.0	21.01	19.7
7	20	170.0	54.4	18.81	17.9
8	34	162.0	55.1	21.00	21.7
9	34	166.0	58.5	21.23	21.9
10	25	165.0	57.0	20.94	21.5
11	25	172.0	67.0	22.65	20.4
12	34	162.0	52.4	19.97	22.3
13	17	171.0	66.5	22.74	22.8
14	34	161.0	57.0	21.99	21.9
15	25	168.0	58.2	20.62	23.2
16	30	170.0	68.0	23.53	23.8
17	21	158.0	53.0	21.23	21.4

Amenorrhoeic and Oligomenorrhoeic Young Athletes

EAT26 & BITE scores

Number of Athlete	EAT26 score	Factor 1 score	Factor 3 score	BITE score
Amenorrhoeic Group				
1	9	7	1	13
2	3	2	1	2
3	5	5	0	10
4	11	7	4	4
5	22	17	3	11
6	4	2	2	3
7	8	5	3	7
8	14	7	7	3
9	16	12	3	10
10	18	13	2	16
11	26	18	5	18
12	2	1	1	4
13	36	18	12	9
14	*	*	*	*
15	*	*	*	*
16	21	15	6	5
17	2	2	0	4
18	20	1	4	15
19	18	15	2	10
20	17	13	4	13
21	12	6	3	14
22	3	2	1	5
23	1	0	1	6
24	3	1	2	7
Oligomenorrhoeic Group				
1	6	4	2	4
2	2	2	0	5
3	4	2	2	8
4	14	12	2	8
5	18	12	6	9
6	21	12	8	21
7	23	17	5	30
8	2	2	0	4
9	4	4	0	15

*denotes incomplete data

Eumenorrhoeic Young Athletes

EAT26 & BITE scores

Number of Athlete	EAT26 score	Factor 1 score	Factor 3 score	BITE score
1	15	9	4	13
2	6	3	3	2
3	1	1	0	3
4	4	2	2	4
5	*	*	*	*
6	7	5	0	6
7	2	0	2	1
8	23	15	2	11
9	6	5	1	2
10	1	1	0	6
11	3	3	0	7
12	2	2	0	3
13	1	1	0	1
14	8	5	3	3
15	1	1	0	9
16	2	2	0	10
17	7	6	0	13

*denotes incomplete data

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Menstrual History

Number of Athlete	Menarche (years)	Years of Eumenorrhoea	Years of Amenorrhoea/ Oligomenorrhoea
Amenorrhoeic Group			
1	15.0	2.0	17.0
2	16.0	2.0	9.0
3	16.1	1.0	1.6
4	13.7	8.0	10.8
5	15.5	2.0	1.5
6	12.5	8.0	17.3
7	15.0	1.5	7.5
8	13.5	2.8	4.8
9	13.2	9.0	8.0
10	13.0	12.0	4.5
11	13.8	8.0	21.2
12	13.0	8.0	12.0
13	13.0	11.0	1.5
14	12.0	12.0	5.0
15	14.0	0.0	12.0
16	13.3	10.0	11.0
17	14.5	5.5	4.2
18	16.0	0.6	6.0
19	13.0	15.0	3.0
20	12.0	11.0	5.0
21	14.0	10.0	7.0
22	13.0	12.0	6.0
23	12.0	1.5	5.0
24	15.0	0.0	5.0
Oligomenorrhoeic Group			
1	14.0	12.0	1.5
2	13.3	3.0	1.5
3	12.0	9.0	12.0
4	17.0	2.0	3.0
5	11.5	15.0	8.0
6	15.0	1.5	3.0
7	14.0	12.0	3.0
8	16.3	2.0	3.0
9	14.2	7.3	9.0

Eumenorrhoeic Young Athletes

Menstrual History

Number of Athlete	Menarche (years)	Years of Eumenorrhoea	Years of Amenorrhoea
1	20.0	5.5	0.0
2	13.0	3.5	0.0
3	13.0	12.0	0.0
4	13.0	10.0	0.0
5	14.2	4.0	0.0
6	13.3	17.0	0.0
7	12.5	7.0	0.0
8	11.3	22.0	0.0
9	18.0	16.0	0.0
10	12.8	13.0	0.0
11	15.0	10.0	0.0
12	13.2	21.0	0.0
13	16.3	1.0	0.0
14	14.5	22.5	0.0
15	13.0	9.0	0.0
16	14.0	16.0	0.0
17	12.6	9.0	0.0

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Training Characteristics

Number of Athlete	Age began training	Miles run per Week	VO_2max ($ml.kg^{-1}.min^{-1}$)
Amenorrhoeic Group			
1	16.0	32	59.6
2	19.0	80	injured
3	14.5	25	57.7
4	22.0	85	63.1
5	15.0	50	65.3
6	32.0	70	58.3
7	25.0	28	43.9
8	18.0	60	70.8
9	21.5	35	39.9
10	26.0	40	55.5
11	16.0	30	49.0
12	27.0	30	54.4
13	15.0	48	injured
14	25.0	75	61.2
15	12.0	90	60.7
16	32.0	80	59.4
17	18.0	45	59.0
18	18.0	30	57.6
19	24.0	80	61.1
20	19.0	55	61.0
21	21.0	60	66.0
22	22.0	65	53.5
23	11.0	50	65.8
24	15.0	60	65.9
Oligomenorrhoeic Group			
1	19.0	25	57.2
2	14.0	50	61.6
3	21.0	38	56.3
4	17.0	63	63.4
5	29.0	55	50.2
6	17.0	35	46.1
7	25.0	70	65.4
8	16.0	50	69.0
9	28.0	40	51.7

Eumenorrhoeic Young Athletes

Training Characteristics

Number of Athlete	Age began training	Miles run per Week	VO_2max ($ml.kg^{-1}.min^{-1}$)
1	17.5	35	59.6
2	12.5	30	59.2
3	14.0	50	63.8
4	21.0	70	69.7
5	14.0	25	59.0
6	15.0	85	64.5
7	12.0	40	58.5
8	32.0	30	58.5
9	29.0	45	60.3
10	23.0	35	52.8
11	22.0	30	55.7
12	23.0	60	62.2
13	13.0	55	50.5
14	29.0	50	50.5
15	14.0	35	injured
16	24.0	50	56.2
17	18.0	30	50.5

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Bone Density of Neck of Femur

Number of Athlete	g.cm^{-2}	Specified Density	T-score (SD)	Z-score (SD)
Amenorrhoeic Group				
1	0.763	0.824	-1.320	-0.978
2	0.646	0.681	-2.490	-2.294
3	0.842	0.925	-0.530	-0.316
4	0.871	0.963	-0.240	0.177
5	0.814	0.889	-0.910	-0.637
6	0.808	0.881	-0.870	-0.341
7	0.796	0.866	-0.990	-0.611
8	0.801	0.872	-0.940	-0.707
9	0.706	0.754	-1.890	-1.543
10	0.742	0.798	-1.530	-1.100
11	0.855	0.942	-0.400	-0.062
12	0.772	0.836	-1.230	-0.736
13	0.801	0.872	-0.940	-0.581
14	0.946	1.064	0.510	0.853
15	0.744	0.801	-1.510	-1.183
16	0.802	0.874	-0.930	-0.424
17	0.770	0.833	-1.250	-0.946
18	0.837	0.919	-0.580	-0.323
19	0.711	0.760	-1.840	-1.445
20	0.769	0.832	-1.260	-0.894
21	0.782	0.848	-1.130	-0.694
22	0.968	1.095	0.730	1.070
23	0.856	0.944	-0.390	-0.199
24	0.913	1.019	0.180	0.362
Oligomenorrhoeic Group				
1	0.794	0.864	-1.010	-0.653
2	0.816	0.892	-0.790	-0.617
3	1.099	1.282	2.040	2.136
4	0.974	1.103	0.790	0.934
5	0.819	0.896	-0.760	-0.230
6	0.813	0.888	-0.820	-0.648
7	0.803	0.875	-0.920	-0.518
8	0.816	0.892	-0.790	-0.596
9	0.672	0.712	-2.230	-1.855

SD = Standard deviations

Eumenorrhoeic Young Athletes

Bone Density of Neck of Femur

Eumenorrhoeic Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	T-score (SD)	Z-score (SD)
1	0.936	1.051	0.410	0.741
2	1.115	1.306	2.200	2.013
3	0.960	1.084	0.650	1.020
4	0.994	1.131	0.990	1.151
5	0.801	0.872	-0.940	-0.770
6	0.859	0.948	-0.360	0.103
7	1.025	1.175	1.300	1.355
8	0.952	1.073	0.570	1.012
9	0.931	1.044	0.360	0.822
10	0.857	0.945	-0.380	-0.064
11	1.146	1.353	2.510	2.448
12	0.956	1.078	0.610	1.048
13	1.018	1.165	1.230	1.232
14	0.773	0.837	-1.220	-0.725
15	0.928	1.040	0.330	0.605
16	1.138	1.340	2.430	2.490
17	1.126	1.322	2.310	2.205

SD = Standard deviations

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Bone Density of Trochanteric Region of Femur

Number of Athlete	$g.cm^{-2}$	Specified Density	T-score (SD)	Z-score (SD)
Amenorrhoeic Group				
1	0.697	0.680	-0.280	-0.683
2	0.645	0.680	-0.850	-0.683
3	0.978	1.108	-0.490	2.581
4	0.601	0.628	-1.340	-1.214
5	0.782	0.848	0.670	0.795
6	0.636	0.669	-0.960	-0.789
7	0.620	0.650	-1.130	-0.981
8	0.678	0.719	-0.490	-0.304
9	0.541	0.558	-2.010	-1.996
10	0.506	0.519	-2.400	-2.488
11	0.680	0.722	-0.470	-0.282
12	0.523	0.538	-2.210	-2.246
13	0.640	0.674	-0.910	-0.742
14	0.646	0.681	-0.840	-0.671
15	0.625	0.656	-1.080	-0.920
16	0.704	0.751	-0.200	-0.017
17	0.581	0.605	-1.570	-1.467
18	0.695	0.740	-0.300	-0.116
19	0.580	0.603	-1.580	-1.480
20	0.607	0.635	-1.280	-1.140
21	0.564	0.585	-1.760	-1.688
22	0.774	0.838	0.580	0.715
23	0.861	0.950	1.540	1.553
24	0.769	0.832	0.520	0.664
Oligomenorrhoeic Group				
1	0.665	0.704	-0.630	-0.452
2	0.728	0.781	0.070	0.240
3	0.856	0.944	1.490	1.506
4	0.706	0.754	-0.180	0.005
5	0.709	0.757	-0.140	0.037
6	0.677	0.718	-0.500	-0.316
7	0.737	0.792	0.170	0.335
8	0.765	0.827	0.480	0.624
9	0.681	0.723	-0.460	-0.271

SD = Standard deviations

Eumenorrhoeic Young Athletes

Bone Density of Trochanteric Region of Femur

Eumenorrhoeic Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	T-score (SD)	Z-score (SD)
1	0.771	0.834	0.540	0.685
2	0.838	0.920	1.290	1.338
3	0.831	0.911	1.210	1.272
4	0.888	0.986	1.840	1.799
5	0.678	0.719	-0.490	-0.304
6	0.745	0.802	0.260	0.418
7	0.933	1.047	2.350	2.197
8	0.790	0.858	0.760	0.874
9	0.843	0.927	1.340	1.385
10	0.776	0.841	0.600	0.735
11	0.911	1.017	2.100	2.004
12	0.800	0.871	0.870	0.973
13	0.945	1.063	2.480	2.301
14	0.735	0.789	0.140	0.314
15	0.794	0.864	0.800	0.914
16	0.807	0.880	0.940	1.041
17	0.839	0.921	1.300	1.347

SD = Standard deviations

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Bone Density of Lumbar Spine (L2-L4)

Number of Athlete	g.cm^{-2}	Specified Density	T-score (SD)	Z-score (SD)
Amenorrhoeic Group				
1	0.857	0.945	-2.020	-1.891
2	0.876	0.970	-1.840	-1.682
3	0.894	0.994	-1.680	-1.487
4	1.014	1.159	-0.590	-0.261
5	1.013	1.158	-0.600	-0.271
6	0.936	1.051	-1.300	-1.045
7	0.891	0.990	-1.710	-1.520
8	0.877	0.971	-1.830	-1.671
9	0.860	0.949	-1.990	-1.857
10	0.825	0.903	-2.310	-2.251
11	0.945	1.063	-1.220	-0.952
12	0.813	0.888	-2.420	-2.388
13	0.875	0.969	-1.850	-1.693
14	1.122	1.316	0.390	0.755
15	0.762	0.823	-2.880	-2.993
16	0.991	1.127	-0.800	-0.488
17	0.770	0.833	-2.800	-2.896
18	1.002	1.142	-0.700	-0.379
19	0.878	0.973	-1.830	-1.660
20	0.855	0.942	-2.030	-1.913
21	0.876	0.970	-1.850	-1.682
22	1.054	1.216	-0.230	0.123
23	1.104	1.290	0.220	0.590
24	0.954	1.075	-1.130	-0.860
Oligomenorrhoeic Group				
1	1.000	1.139	-0.730	-0.399
2	0.867	0.958	-1.930	-1.780
3	1.088	1.266	0.080	0.443
4	0.922	1.032	-1.430	-1.191
5	1.000	1.139	-0.350	-0.399
6	0.910	1.015	-1.540	-1.317
7	1.009	1.152	-0.640	-0.310
8	0.961	1.085	-1.080	-0.789
9	1.044	1.202	-0.320	0.028

SD = Standard deviations

Eumenorrhoeic Young Athletes

Bone Density of Lumbar Spine (L2-L4)

Eumenorrhoeic Athletes

Number of Athlete	g.cm^{-2}	Specified Density	T-score (SD)	Z-score (SD)
1	1.070	1.239	-0.070	0.275
2	1.100	1.284	0.140	0.554
3	1.203	1.441	1.120	1.474
4	1.210	1.452	1.190	1.535
5	0.840	0.923	-2.170	-2.080
6	1.141	1.345	0.560	0.927
7	1.154	1.365	0.680	1.043
8	1.089	1.267	0.090	0.452
9	1.136	1.337	0.510	0.882
10	1.084	1.260	0.050	0.405
11	1.034	1.187	-0.410	-0.068
12	1.046	1.205	-0.300	0.047
13	1.039	1.195	-0.360	-0.020
14	1.207	1.447	1.160	1.509
15	1.139	1.342	0.540	0.909
16	1.039	1.195	-0.360	-0.020
17	0.947	1.066	-1.200	-0.932

SD = Standard deviations

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Dietary Calcium intake and Biochemical Parameters

No. of Athlete	Dietary Calcium (mg)	Serum Calcium ($mmol.^{-1}$)	Serum Phosphate ($mmol.^{-1}$)	Serum Osteocalcin ($ng.ml^{-1}$)	Serum Alkaline Phosphatase ($mmol.^{-1}$)
1	1215	2.37	1.45	2.0	54
2	741	*	*	*	*
3	444	2.25	1.42	0.8	57
4	1118	2.37	1.40	1.5	67
5	1134	2.21	1.28	2.0	43
6	1009	2.28	1.24	2.2	58
7	1935	2.23	1.42	2.7	53
8	1355	2.45	1.23	1.1	35
9	587	2.25	1.18	2.8	109
10	398	2.39	1.10	2.4	69
11	342	2.31	1.18	3.7	72
12	327	2.35	1.17	3.2	77
13	400	2.29	1.12	1.4	51
14	601	2.31	1.37	4.4	69
15	933	2.45	1.24	3.5	46
16	981	2.21	1.13	2.8	49
17	768	2.32	1.35	2.6	70
18	706	2.40	1.26	2.8	55
19	962	2.33	1.03	3.4	85
20	877	2.33	1.30	2.8	63
21	336	2.31	1.39	1.2	75
22	630	2.38	1.11	1.7	54
23	942	2.20	1.42	3.4	61
24	1157	2.34	1.19	1.8	71
Oligomenorrhoeic Group					
1	476	2.23	0.90	3.2	63
2	1063	*	*	*	*
3	590	2.36	0.86	3.4	59
4	186	2.31	1.24	3.5	75
5	583	2.29	1.19	2.8	37
6	489	2.34	1.26	4.4	57
7	1814	2.29	1.19	1.1	49
8	1066	2.37	1.11	1.2	63
9	1002	2.31	1.02	1.4	47

Eumenorrhoeic Young Athletes

Dietary Calcium intake and Biochemical Parameters

Eumenorrhoeic Athletes

Number of Athlete	Dietary Calcium (mg)	Serum Calcium ($mmol.^{-l}$)	Serum Phosphate ($mmol.^{-l}$)	Serum Osteocalcin ($ng.ml^{-1}$)	Serum Alkaline Phosphatase ($mmol.^{-l}$)
1	1380	2.43	1.11	3.1	60
2	327	2.39	1.29	4.8	67
3	621	2.22	0.92	2.8	65
4	890	2.33	1.10	2.6	39
5	1007	2.25	1.39	5.8	69
6	573	2.20	1.26	2.6	60
7	2107	2.23	1.01	3.9	125
8	1738	2.18	0.98	3.8	49
9	1212	2.41	1.21	4.3	66
10	1041	2.31	1.31	3.3	59
11	1015	2.31	1.08	3.5	44
12	500	2.29	1.37	2.2	60
13	1364	2.36	1.23	5.9	120
14	1131	2.25	1.22	2.2	39
15	1073	2.41	1.48	3.2	37
16	1010	2.28	1.03	3.5	46
17	958	2.49	1.15	2.3	54

Amenorrhoeic and Oligomenorrhoeic Young Athletes

Serum hormone measurements

Oestrogen ($pg.ml^{-1}$)	FSH ($mIU.ml^{-1}$)	PRL ($mU.ml^{-1}$)
Amenorrhoeic group		
157	7.1	218
*	*	*
115	7.9	403
143	8.7	134
75	3.2	127
70	3.0	94
157	0.5	134
70	1.6	209
70	5.5	113
134	9.1	106
97	7.6	228
174	9.0	185
654	3.5	
73	5.7	91
77	0.8	394
70	4.8	146
169	7.5	161
127	8.1	173
737	5.9	158
165	4.4	166
74	5.0	67
75	4.5	94
90	5.4	314
137	7.8	187
Oligomenorrhoeic Group		
820	5.2	125
70	1.1	115
*	*	*
166	6.3	139
80	8.5	82
931	8.1	310
368	3.2	142
140	6.0	156
76	2.3	296

Key

FSH: follicle stimulating hormone
PRL: prolactin
*: data not available

Eumenorrhoeic Young Athletes

Serum hormone measurements

Eumenorrhoeic Group

Oestrogen ($pg.ml^{-1}$)	FSH ($mIU.ml^{-1}$)	PRL ($mU.ml^{-1}$)
935	5.2	175
253	5.5	120
455	6.3	106
494	2.3	175
77	3.1	1066
243	6.4	386
255	2.7	314
404	6.7	125
225	8.9	444
163	5.5	211
323	3.2	334
428	12.1	938
70	2.3	161
157	5.3	430
198	5.2	202
143	3.9	622
716	2.1	156

Key

FSH:	follicle stimulating hormone
PRL:	prolactin
*	data not available

Additional statistical analysis of data for athletes aged 16 - 35 years

Table 1

Linear regression of BMD ($g.cm^{-2}$) with possible independent variables

Factor	Trochanteric		Neck of Femur		Lumbar Spine	
	F	p	F	p	F	p
Age	9.13	**0.004**	3.30	0.08	0.38	0.54
Height	4.31	**0.043**	3.63	0.06	6.95	**0.011**
Weight	6.45	**0.014**	10.40	**0.002**	10.60	**0.002**
BMI	3.01	0.09	5.83	**0.02**	4.38	**0.04**
% fat	0.19	0.67	1.57	0.22	3.17	0.081
Menarche	0.91	0.34	0.11	0.75	0.32	0.57
Yrs. Eumen	0.01	0.94	1.01	0.32	11.08	**<0.002**
Mpw	1.89	0.18	1.64	0.21	0.03	0.87
Age Began	4.56	**0.038**	1.07	0.31	0.39	0.53
Diet Ca	3.77	0.06	0.22	0.64	2.12	0.15
VO2max	1.02	0.32	0.14	0.71	0.80	0.38
EAT 26	8.33	**0.006**	4.87	**0.03**	4.14	**0.048**
Factor 1	7.32	**0.01**	5.02	**0.03**	4.85	**0.03**
Factor 2	2.48	0.12	0.67	0.42	0.97	0.33
Factor 3	5.92	**0.019**	3.11	0.09	2.28	0.14
BITE	6.24	**0.016**	2.74	0.11	2.38	0.13
Oestrogen	0.34	0.56	1.40	0.24	0.71	0.41

KEY

BMI: body mass index
Yrs Eumen: years of eumenorrhoea
Mpw: miles run per week
Age began: age at whoch began training
Diet Ca: dietary calcium intake

Table 5

Linear regression within individual menstrual groups

BMD ($g.cm^{-2}$)

Neck of Femur

	FN vs. Height		FN vs. Weight	
	F	**p**	**F**	**p**
AM	16.30	0.001	4.02	0.06
OL	0.32	0.59	0.34	0.58
EU	0.16	0.70	1.22	0.29

Trochanteric Region

	FT vs. Height		FT vs. Weight	
	F	**p**	**F**	**p**
AM	4.02	0.06	0.18	0.68
OL	0.85	0.39	1.55	0.26
EU	1.89	0.16	1.05	0.32

Lumbar Spine

	L2 - L4 vs. Height		L2 - L4 vs. Weight	
	F	**p**	**F**	**p**
AM	14.07	0.001	6.43	0.019
OL	0.03	0.87	1.42	0.28
EU	0.12	0.73	0.002	0.96

Table 2

Multiple regression analysis of BMD ($g.cm^{-2}$) of neck of femur

Variable	Stage 1		Stage 2	
	$F_{1,48}$	P-value	$F_{3,46}$	P-value
Age	3.30	0.08	3.90	NS
Height	3.63	0.06	2.33	NS
Weight	10.41	0.002	1.28	NS
BMI	5.83	0.02	0.14	NS
EAT26 score	4.87	0.03	0.52	NS
Factor 1	5.02	0.03	0.97	NS
Menstrual Status	**15.069**	**<0.005**		

Menstrual status added in at stage 2.

Table 3

Multiple regression analysis of BMD ($g.cm^{-2}$) of trochanteric region

Variable	Stage 1		Stage 2		Stage 3	
	$F_{1,48}$	p	$F_{1,46}$	p	$F_{1,45}$	p
Age	9.13	0.004	**12.18**	**<0.01**		
Height	4.31	0.04	3.32	NS		
Weight	6.45	0.014	0.02	NS		
Years of Eumenorrhoea	0.01	0.94	7.21	<0.05	0.09	NS
Age began training	4.56	0.04	5.92	<0.05	0.07	NS
EAT26 score	8.33	0.01	2.54	NS		
Factor 1	7.32	0.01	2.64	NS		
Factor 3	5.92	0.02	1.10	NS		
BITE	6.24	0.02	0.50	NS		
Menstrual Status	**15.69**	**<0.001**				

Variables added in at each stage shown in bold

Table 4

Multiple regression analysis of BMD ($g.cm^{-2}$) of lumbar spine

Variable	Stage 1		Stage 2	
	$F_{1,49}$	P-value	$F_{1,48}$	P-value
Height	6.95	0.01	**6.40**	**<0.05**
Weight	10.60	0.002	1.43	NS
BMI	4.38	0.04	0.05	NS
Years of Eumenorrhoea	11.08	0.002	2.91	NS
EAT26 score	4.14	0.05	0.08	NS
Factor 1	4.85	0.03	0.69	NS
Menstrual Status	**16.098**	**<0.001**		

Menstrual status added in at stage 2

Table 6

Multiple regression analysis of Z-score of neck of femur

Variable	Stage 1		Stage 2	
	$F_{1,48}$	P-value	$F_{3,46}$	P-value
Height	4.09	0.049	3.71	NS
Weight	11.41	0.001	3.01	NS
BMI	5.99	0.018	1.08	NS
EAT26 score	4.33	0.043	0.29	NS
Menstrual Status	**15.069**	**<0.005**		

Menstrual status added in at stage 2.

Table 7

Multiple regression analysis of Z-score of trochanteric region

Variable	Stage 1		Stage 2	
	$F_{1,49}$	P-value	$F_{1,48}$	P-value
Height	4.47	0.040	3.65	NS
Weight	6.50	0.014	0.17	NS
Years of Eumenorrhoea	0.01	0.978	**6.58**	**0.05**
Age began training	4.19	0.046	**5.57**	**0.05**
EAT26 score	7.47	0.009	0.60	NS
Menstrual Status	**15.69**	**<0.001**		

Table 8

Multiple regression analysis of Z-score of lumbar spine

Variable	Stage 1		Stage 2	
	$F_{1,49}$	P-value	$F_{1,48}$	P-value
Height	7.25	0.010	**6.801**	**<0.01**
Weight	11.10	0.002	1.692	NS
BMI	4.50	0.039	0.028	NS
Body Fat (%)	4.38	0.042	0.232	NS
Years of Eumenorrhoea	10.77	0.002	2.847	NS
Menstrual Status	**16.098**	**<0.001**		

APPENDIX 2

DATA AND FURTHER STATISTICAL ANALYSIS FOR PREMENOPAUSAL VETERAN ATHLETES

Premenopausal Veteran Athletes

Demographic Variables of All-Timer Athletes

Number of Athlete	Age (years)	Height (cm)	Weight (kg)	Body Mass Index ($kg.m^{-2}$)
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	48	162.5	60.0	22.72
2	43	160.0	50.0	19.53
3	45	161.3	53.5	20.56
4	42	172.0	60.0	20.28
5	52	164.0	64.5	23.98
6	51	176.0	67.0	21.63
7	40	163.4	56.7	21.24
8	40	160.5	64.5	25.04
9	41	166.0	59.5	21.59
10	44	171.0	63.0	21.55
11	48	172.0	60.0	20.28
12	44	164.0	63.2	23.50
13	40	167.5	62.1	22.13
14	55	165.0	53.8	19.76
15	50	163.0	54.5	20.51
Group Ab - Always Athletic, Irregular Menstruation				
1	41	163.0	59.0	22.21
2	40	159.0	49.5	19.58
3	42	164.0	60.0	22.31
4	40	168.0	51.0	18.07
5	43	162.0	56.7	21.60
6	44	157.0	47.9	19.43
7	42	171.0	63.3	21.65
8	48	164.0	55.5	20.64

Premenopausal Veteran Athletes

Demographic Variables of Late-Starter Athletes

Number of Athlete	Age (years)	Height (cm)	Weight (kg)	Body Mass Index ($kg.m^{-2}$)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	48	163.3	61.2	22.95
2	45	163.0	58.5	22.02
3	43	165.0	52.0	19.10
4	46	167.3	58.5	20.90
5	48	164.0	53.5	19.89
6	49	165.0	60.5	22.22
7	45	161.0	60.5	23.34
8	47	162.5	56.9	21.53
9	43	161.4	74.4	28.56
10	45	155.0	47.6	19.81
11	47	158.0	53.0	21.23
12	44	165.0	63.3	23.25
13	48	159.0	58.4	23.10
14	55	161.0	62.0	23.92
15	46	153.0	47.3	20.21
16	50	166.5	54.5	19.66
17	40	164.0	54.0	20.08
18	44	159.0	54.5	21.56
19	46	163.3	55.8	20.92
20	44	160.7	58.2	22.54
21	42	154.8	54.2	22.62
22	46	164.5	60.6	22.39
Group Bb - Late-starter, Irregular Menstruation				
1	50	160.5	50.4	19.57
2	41	162.5	53.6	20.30
3	40	166.0	61.5	22.32
4	44	163.0	62.0	23.34
5	41	172.0	64.0	21.63

*denotes incomplete data

Premenopausal Veteran Athletes

Body Composition Details of All-Timer Athletes

Number of Athlete	Body Fat 1 (%)	Total Body Potassium (mmol)	Fat Free Mass (kg)	Body Fat (kg)	Body Fat 2 (%)	Fat Free Mass (%)
Group Aa - Always Athletic, Always Eumenorrhoeic						
1	29.5	2664.0	44.4	15.6	26.00	74.0
2	24.9	2502.0	41.7	8.3	16.60	83.4
3	25.4	2706.0	45.1	8.4	15.70	84.3
4	27.1	2892.0	48.2	11.8	19.67	80.3
5	26.5	3026.9	50.5	14.0	21.71	78.3
6	21.4	3138.0	52.3	13.7	20.45	78.1
7	26.1	2703.6	45.1	11.6	20.46	79.5
8	28.9	2760.0	46.0	18.5	28.68	71.3
9	24.3	3042.0	50.7	8.8	14.79	85.2
10	24.1	2830.9	47.2	15.8	25.08	74.9
11	24.5	2958.0	49.3	10.7	17.83	82.2
12	27.3	2889.8	48.2	14.0	22.15	76.3
13	24.1	2880.0	48.0	14.1	22.71	77.3
14	25.7	2570.6	42.8	22.5	20.37	79.6
15	24.9	2574.0	42.9	11.1	20.37	78.7
Group Ab - Always Athletic, Irregular Menstruation						
1	24.8	2784.0	46.4	12.6	21.36	78.6
2	24.1	2310.0	38.5	11.5	23.23	77.8
3	23.9	3210.0	53.5	6.5	10.83	89.2
4	16.1	2754.0	45.9	5.1	10.00	90.0
5	29.3	2442.0	40.7	16.3	28.75	71.8
6	20.4	2313.0	38.6	9.3	19.42	80.6
7	24.2	3054.0	50.9	12.4	19.59	80.4
8	26.5	2754.0	45.9	9.6	17.30	82.7

Premenopausal Veteran Athletes

Body Composition Details of Late-Starter Athletes

Number of Athlete	Body Fat 1 (%)	Total Body Potassium (mmol)	Fat Free Mass (kg)	Body Fat (kg)	Body Fat 2 (%)	Fat Free Mass (%)
Group Ba - Late-starter, Always Eumenorrhoeic						
1	28.0	2752.2	45.9	0.0	25.05	75.0
2	25.8	2652.0	44.2	14.3	24.44	75.6
3	18.6	2568.0	42.8	9.2	17.69	82.3
4	25.1	3024.0	50.4	8.1	13.85	86.2
5	29.9	2706.0	45.1	8.4	15.70	84.3
6	28.8	3066.0	51.1	9.4	15.54	84.5
7	30.2	2520.0	42.0	18.5	30.58	69.4
8	28.2	*	*	*	*	*
9	31.4	2979.0	49.7	0.0	33.27	66.7
10	24.2	2310.0	38.5	8.5	17.86	80.9
11	24.5	2652.0	44.2	8.8	16.60	83.4
12	24.1	2724.0	45.4	7.9	12.48	71.7
13	29.2	2490.0	41.5	16.5	28.25	71.1
14	36.3	2202.0	36.7	25.3	40.81	59.2
15	20.3	2298.0	38.3	9.0	19.03	81.0
16	25.0	2568.0	42.8	11.7	21.47	78.5
17	22.4	2628.0	43.8	10.0	18.52	81.1
18	23.2	2863.9	47.7	21.7	12.42	87.6
19	28.5	2760.0	46.0	9.8	17.56	82.4
20	31.0	2292.0	38.2	20.0	34.36	65.6
21	32.5	2398.2	40.0	4.4	26.25	73.7
22	25.3	2800.0	46.7	3.2	22.99	77.0
Group Bb - Late-starter, Irregular Menstruation						
1	28.9	2124.0	35.4	15.0	29.76	70.2
2	23.4	2592.0	43.2	10.4	19.40	80.6
3	32.1	2761.0	46.0	15.5	25.20	74.8
4	31.9	2909.7	48.5	22.4	21.78	78.2
5	30.2	2538.0	42.3	21.7	33.91	66.1

*denotes incomplete data

Premenopausal Veteran Athletes

Gynaecological History of All-Timer Athletes

Number of Athlete	Age at Menarche (years)	No. Years EU	No. Years OL/AM	No. Years PM	HRT use (years)
Group Aa - Always Athletic, Always Eumenorrhoeic					
1	13.0	35.5	0.5	1.0	1.0
2	14.0	29.0	0.0	0.0	0.0
3	11.8	34.0	0.0	0.0	0.0
4	12.5	30.0	0.0	0.0	0.0
5	14.0	38.0	0.0	0.0	0.0
6	14.8	36.0	0.0	0.0	0.0
7	12.5	27.5	0.0	0.0	0.0
8	13.0	27.0	0.0	0.0	0.0
9	13.0	28.0	0.0	0.0	2.0
10	14.0	30.0	0.0	0.0	0.0
11	17.0	29.0	0.0	0.0	0.0
12	11.8	32.3	0.0	0.0	0.0
13	12.0	29.0	0.0	0.0	0.0
14	15.0	40.0	0.5	3.0	3.0
15	13.8	37.0	0.0	0.0	0.0
Group Ab - Always Athletic, Irregular Menstruation					
1	15.0	13.0	12.0	0.0	0.0
2	14.5	17.0	4.0	0.0	0.0
3	13.0	22.0	6.0	0.0	0.0
4	12.0	6.0	22.0	0.0	0.0
5	12.0	13.0	16.0	5.0	5.0
6	12.5	12.5	16.0	0.0	0.0
7	12.0	26.0	5.0	0.0	0.0
8	14.0	12.0	16.0	0.0	0.0

KEY

HRT: hormone replacement therapy
EU: eumenorrhoea
OL/AM: oligomenorrhoea or amenorrhoea
PM: postmenopause

Premenopausal Veteran Athletes

Gynaecological History of Late-Starter Athletes

Number of Athlete	Age at Menarche (years)	No. Years EU	No. Years OL/AM	No. Years PM	HRT use (years)
Group Ba - Late-starter, Always Eumenorrhoeic					
1	13.2	35.0	0.0	0.0	0.0
2	13.0	32.0	0.0	0.0	0.0
3	13.0	30.0	0.0	0.0	0.0
4	15.0	30.0	0.8	1.5	0.8
5	16.0	32.0	0.0	0.0	0.0
6	12.5	37.0	0.0	3.0	3.0
7	13.0	32.0	0.0	0.0	0.0
8	13.0	34.0	0.0	0.0	0.0
9	15.0	28.0	0.0	0.0	0.0
10	11.5	34.0	0.0	1.0	1.0
11	10.0	37.0	0.0	0.0	0.0
12	16.0	28.0	0.0	0.0	0.0
13	12.5	36.0	0.0	1.0	1.0
14	13.0	42.0	0.0	8.0	8.0
15	13.0	33.0	0.5	0.0	0.0
16	13.0	37.0	0.0	4.0	4.0
17	15.5	24.5	0.0	0.0	0.0
18	12.0	32.0	0.0	0.0	0.0
19	15.0	31.0	0.0	0.0	0.0
20	12.6	31.5	0.0	0.0	0.0
21	13.0	29.0	0.0	0.0	0.0
22	11.5	35.0	0.0	0.0	0.0
Group Bb - Late-starter, Irregular Menstruation					
1	16.2	28.0	6.0	7.0	1.0
2	15.0	20.0	6.0	0.0	0.0
3	13.0	30.0	6.0	0.0	0.0
4	12.3	26.0	6.0	0.0	0.0
5	12.0	27.0	2.0	0.0	0.0

KEY

HRT: hormone replacement therapy
EU: eumenorrhoea
OL/AM: oligomenorrhoea or amenorrhoea
PM: postmenopause

Premenopausal Veteran Athletes

Obstetric History of All-Timer Athletes

Number of Athlete	OCP use (years)	Pregnancies	Breast fed (months)
Group Aa - Always Athletic, Always Eumenorrhoeic			
1	1.0	3	22.0
2	0.0	0	0.0
3	14.0	0	0.0
4	2.0	2	7.0
5	4.0	3	9.0
6	0.0	0	0.0
7	10.0	1	5.0
8	3.0	2	12.0
9	21.0	2	24.0
10	1.0	2	6.0
11	10.0	0	0.0
12	1.0	2	1.8
13	0.0	0	0.0
14	0.0	2	8.0
15	0.5	2	11.0
Group Ab - Always Athletic, Irregular Menstruation			
1	0.5	2	36.0
2	9.0	1	0.0
3	0.0	0	0.0
4	1.0	2	10.0
5	0.7	0	0.0
6	4.0	3	6.0
7	7.0	2	10.0
8	10.0	1	0.8

KEY

OCP Oral contraceptive Pill

Premenopausal Veteran Athletes

Obstetric History of Late-Starter Athletes

Number of Athlete	OCP use (years)	Pregnancies	Breast fed (months)
Group Ba - Late-starter, Always Eumenorrhoeic			
1	4.0	3	3.0
2	0.5	2	0.0
3	5.0	1	10.0
4	3.0	2	8.5
5	15.0	1	0.0
6	1.5	2	4.0
7	10.0	0	0.0
8	6.0	3	27.0
9	3.0	1	0.0
10	5.0	4	0.5
11	9.0	2	2.0
12	11.0	2	0.0
13	0.0	3	1.5
14	0.0	2	13.0
15	1.0	2	5.0
16	25.0	2	20.0
17	11.0	3	4.0
18	5.0	2	1.5
19	0.0	0	0.0
20	1.0	2	0.0
21	5.0	2	15.0
22	12.0	1	4.0
Group Bb - Late-starter, Irregular Menstruation			
1	7.0	3	2.0
2	9.0	1	12.0
3	4.0	2	12.0
4	21.0	3	0.0
5	1.0	3	18.5

KEY

OCP Oral contraceptive Pill

Premenopausal Veteran Athletes

Training Details of All-Timer Athletes

Number of Athlete	Distance Run Per Week (miles)	Time Trained Per Week (hours)	Sessions per week
Group Aa - Always Athletic, Always Eumenorrhoeic			
1	10	3.0	3.0
2	30	7.0	7.0
3	12	2.0	2.0
4	75	10.0	7.0
5	40	14.0	10.0
6	40	7.0	7.0
7	30	5.0	5.0
8	20	5.0	5.0
9	15	5.0	6.0
10	33	7.5	6.0
11	20	9.0	6.0
12	20	5.0	5.0
13	5	2.0	2.0
14	40	17.5	10.0
15	15	4.0	4.0
Group Ab - Always Athletic, Irregular Menstruation			
1	17	4.0	4.0
2	50	12.0	12.0
3	45	9.0	6.0
4	50	8.0	7.0
5	20	4.0	5.0
6	52.5	19.0	15.0
7	35	8.0	8.0
8	40	6.0	6.0

Premenopausal Veteran Athletes

Training Details of Late-Starter Athletes

Number of Athlete	Distance Run Per Week (miles)	Time Trained Per Week (hours)	Sessions per week
Group Ba - Late-starter, Always Eumenorrhoeic			
1	30	6.0	5.0
2	30	7.0	9.0
3	10	5.0	7.0
4	30	11.0	8.0
5	30	9.0	9.0
6	40	7.0	5.0
7	50	9.0	6.0
8	12	6.0	6.0
9	25	5.0	5.0
10	25	5.0	5.0
11	40	7.0	6.0
12	60	11.0	8.0
13	10	10.0	8.0
14	12	4.0	4.0
15	50	9.0	6.0
16	40	7.8	9.0
17	60	10.0	8.0
18	35	4.0	6.0
19	25	6.0	6.0
20	25	6.0	7.0
21	25	4.0	4.0
22	25	5.0	8.0
Group Bb - Late-starter, Irregular Menstruation			
1	33	5.0	5.0
2	35	5.5	6.0
3	40	5.0	5.0
4	30	8.5	9.0
5	18	5.0	5.0

Premenopausal Veteran Athletes

Maximal Oxygen Uptake and Grip Strength of

Number of Athlete	VO_2max ($ml.kg^{-1}.min^{-1}$)	Grip Strength Dominant Hand (Kgf)	Grip Strength Non-Dominant Hand (Kgf)
Group Aa - Always Athletic, Always Eumenorrhoeic			
1	42.7	34.5	28.5
2	59.3	32.0	26.5
3	48.1	30.0	24.5
4	50.2	42.5	40.0
5	44.7	30.0	33.0
6	37.1	32.0	27.0
7	46.1	37.0	34.0
8	34.8	38.5	38.0
9	53.1	38.5	36.0
10	50.7	39.0	33.5
11	40.3	48.5	48.5
12	46.7	32.5	30.5
13	39.3	46.5	42.0
14	54.2	32.5	31.0
15	43.2	41.0	36.0
Group Ab - Always Athletic, Irregular Menstruation			
1	44.8	31.0	30.0
2	55.7	30.5	26.0
3	52.2	35.5	34.0
4	63.1	34.5	34.0
5	40.0	36.5	34.0
6	53.4	28.0	29.5
7	44.7	36.0	33.5
8	53.1	34.5	32.0

Premenopausal Veteran Athletes

Maximal Oxygen Uptake and Grip Strength of Late-Starter Athletes

Number of Athlete	VO_2max ($ml.kg^{-1}.min^{-1}$)	Grip Strength Dominant Hand (Kgf)	Grip Strength Non-Dominant Hand (Kgf)
Group Ba - Late-starter, Always Eumenorrhoeic			
1	51.6	37.0	33.0
2	48.2	40.0	35.0
3	51.0	37.0	32.0
4	43.7	39.5	36.5
5	46.5	27.0	22.0
6	41.4	35.0	31.5
7	41.0	29.0	27.5
8	38.8	32.0	30.5
9	40.1	40.0	37.0
10	44.0	29.5	24.0
11	43.8	40.0	34.5
12	55.1	35.5	31.5
13	36.2	32.5	30.0
14	33.8	37.0	34.0
15	41.6	31.0	24.0
16	52.1	33.5	32.0
17	58.1	33.0	31.5
18	50.9	38.5	36.0
19	50.7	38.0	34.5
20	39.5	27.0	23.0
21	Unwell	32.5	31.0
22	41.8	29.0	28.5
Group Bb - Late-starter, Irregular Menstruation			
1	46.5	26.5	23.5
2	59.3	27.5	28.5
3	28.8	36.0	32.0
4	38.9	38.0	37.0
5	41.3	32.0	29.5

Premenopausal Veteran Athletes

Dietary Calcium Intake and Serum Parameters of Bone Metabolism

Number of Athlete	Calcium Intake ($mg.day^{-1}$)	Serum Calcium ($mmol.l^{-1}$)	Serum Phosphate ($mmol.l^{-1}$)	Alkaline Phosphatase ($mmol.l^{-1}$)
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	1445	2.32	0.94	64
2	322	2.15	1.54	48
3	850	2.47	1.14	56
4	1462	2.18	1.05	67
5	1310	2.14	1.35	47
6	920	2.33	1.18	55
7	1028	2.13	1.34	58
8	1049	2.12	1.66	72
9	794	2.25	1.43	57
10	947	2.20	1.59	54
11	1325	2.20	1.65	86
12	943	2.22	1.17	58
13	976	2.32	1.38	46
14	1005	2.29	1.09	82
15	938	2.27	0.90	41
Group Ab - Always Athletic, Irregular Menstruation				
1	1004	2.23	1.02	52
2	466	2.26	1.09	58
3	780	2.21	1.09	58
4	878	*	*	58
5	93	2.28	1.15	60
6	606	2.31	1.27	100
7	893	2.21	1.07	56
8	1003	2.25	1.29	49

*denotes incomplete data

Serum calcium corrected for serum albumin

Premenopausal Veteran Athletes

Dietary Calcium Intake and Serum Parameters of Bone Metabolism in Late-Starter Athletes

Number of Athlete	Calcium Intake ($mg.day^{-1}$)	Serum Calcium ($mmol.l^{-1}$)	Serum Phosphate ($mmol.l^{-1}$)	Alkaline Phosphatase ($mmol.l^{-1}$)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	1150	2.22	1.05	66
2	486	2.25	1.08	57
3	1409	2.21	1.07	47
4	1186	2.25	1.31	46
5	616	2.20	1.24	65
6	1403	2.35	1.24	96
7	1389	2.29	1.37	61
8	980	2.30	1.21	46
9	1059	2.39	1.10	50
10	768	2.36	1.25	67
11	1083	2.31	1.34	59
12	1141	2.23	1.67	78
13	N/A	2.16	1.18	46
14	559	2.37	1.33	55
15	796	2.26	1.22	54
16	779	2.24	0.87	48
17	1831	2.28	1.25	71
18	1011	2.31	1.33	60
19	607	2.16	1.14	64
20	1194	2.27	1.28	87
21	1314	2.31	1.42	63
22	332	2.24	1.06	48
Group Bb - Late-starter, Irregular Menstruation				
1	1000	2.21	1.31	71
2	1106	2.31	1.54	62
3	949	2.32	1.36	71
4	1123	2.20	1.18	58
5	684	2.22	1.23	63

Serum calcium corrected for serum albumin

Premenopausal Veteran Athletes

Back Strength of All-Timer Athletes

Number of Athlete	Peak Torque Extension		Peak Torque Flexion	
	(60o.sec^{-1})	(180o.sec^{-1})	(60o.sec^{-1})	(180o.sec^{-1})
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	197	182	118	107
2	134	113	118	102
3	117	76	144	77
4	176	150	149	155
5	233	237	186	188
6	149	141	126	89
7	156	237	159	198
8	226	220	150	165
9	279	327	165	161
10	152	186	183	175
11	127	171	171	195
12	150	73	119	69
13	267	156	159	175
14	95	144	164	153
15	134	104	133	140
Group Ab - Always Athletic, Irregular Menstruation				
1	205	182	171	144
2	150	108	130	142
3	222	225	167	163
4	171	212	125	121
5	164	186	150	118
6	138	121	108	89
7	164	164	134	84
8	205	161	167	145

Premenopausal Veteran Athletes

Back Strength of Late-Starter Athletes

Number of Athlete	Peak Torque Extension ($60^o.sec^{-1}$)	($180^o.sec^{-1}$)	Peak Torque Flexion ($60^o.sec^{-1}$)	($180^o.sec^{-1}$)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	206	176	142	148
2	174	149	156	136
3	209	201	106	98
4	175	127	168	146
5	148	122	133	100
6	136	123	172	117
7	197	178	146	144
8	161	195	138	132
9	212	197	164	153
10	125	113	106	83
11	174	136	132	103
12	190	190	167	119
13	87	94	126	114
14	180	125	141	126
15	85	60	85	69
16	218	212	134	155
17	current back injury			
18	175	152	117	104
19	183	142	153	152
20	123	133	117	92
21	121	106	99	72
22	155	203	156	171
Group Bb - Late-starter, Irregular Menstruation				
1	129	157	125	104
2	179	149	152	142
3	207	209	149	164
4	175	163	119	133
5	167	133	140	85

Premenopausal Veteran Athletes

Urinary Parameters of Bone Metabolism in All-Timer Athletes

Number of Athlete	Urinary Creatinine ($mmol.l^{-1}$)	Urinary Calcium ($mmol.l^{-1}$)	Urinary OHPR	Ca:creat ratio	OHPR:creat ratio
Group Aa - Always Athletic, Always Eumenorrhoeic					
1	10.6	5.1	81.0	0.5	7.6
2	14.5	11.1	56.0	0.8	3.9
3	7.3	2.3	97.0	0.3	13.3
4	15.3	14.5	79.0	0.9	5.2
5	*	5.8	217.0	*	*
6	3.5	0.8	151.0	0.2	43.1
7	16.2	5.0	279.0	0.3	17.2
8	12.9	6.6	152.0	0.5	11.8
9	14.2	6.1	73.0	0.4	5.1
10	15.2	4.5	133.0	0.3	8.8
11	14.5	4.1	183.0	0.3	12.6
12	18.6	5.3	300.0	0.3	16.1
13	11.4	2.0	76.0	0.2	6.7
14	17.6	3.6	57.0	0.2	3.2
15	13.1	12.8	161.0	1.0	12.3
Group Ab - Always Athletic, Irregular Menstruation					
1	11.3	4.7	254.0	0.4	22.5
2	16.7	7.0	61.0	0.4	3.7
3	15.9	3.0	147.0	0.2	9.2
4	7.5	3.4	113.0	0.5	15.1
5	24.8	6.7	132.0	0.3	5.3
6	4.1	1.9	29.0	0.5	7.1
7	5.1	3.0	58.0	0.6	11.4
8	8.1	8.0	300.0	1.0	37.0

KEY

OHPR	hydroxyproline	*denotes incomplete data
Ca	calcium	
creat	creatinine	

Premenopausal Veteran Athletes

Urinary Parameters of Bone Metabolism in Late-Starter Athletes

Number of Athlete	Urinary Creatinine ($mmol.l^{-1}$)	Urinary Calcium ($mmol.l^{-1}$)	Urinary OHPR	Ca:creat ratio	OHPR:creat ratio
1	4.6	2.0	43.0	0.4	9.3
2	7.3	1.9	92.0	0.3	12.6
3	3.9	1.0	51.0	0.3	13.1
4	5.4	4.0	85.0	0.7	15.7
5	2.9	2.0	33.0	0.7	11.4
6	4.5	1.5	69.0	0.3	15.3
7	7.0	1.1	72.0	0.2	10.3
8	6.3	2.1	40.0	0.3	6.3
9	23.5	11.8	171.0	0.5	7.3
10	12.5	2.4	128.0	0.2	10.2
11	9.5	2.9	130.0	0.3	13.7
12	19.3	6.5	446.0	0.3	23.1
13	12.4	5.8	121.0	0.5	9.8
14	7.3	2.9	80.0	0.4	11.0
15	8.0	0.5	178.0	0.1	22.3
16	2.9	1.6	87.0	0.6	30.0
17	7.2	4.2	161.0	0.6	22.4
18	4.2	3.3	92.0	0.8	21.9
19	10.7	2.3	97.0	0.2	9.1
20	7.3	4.4	86.0	0.6	11.8
21	5.7	5.8	99.0	1.0	17.4
22	4.0	1.8	62.0	0.5	15.5
Group Bb - Late-starter, Irregular Menstruation					
1	13.6	2.1	134.0	0.2	9.9
2	6.7	*	272.0	*	40.6
3	9.8	2.3	155.0	0.2	15.8
4	4.8	2.9	56.0	0.6	11.7
5	7.5	6.4	110.0	0.9	14.7

KEY

OHPR	hydroxyproline	* denotes incomplete data
Ca	calcium	
creat	creatinine	

Premenopausal Veteran Athletes

Sporting Details of All-Timer Athletes

Number of Athlete	Previous sport	Sport now	Level now
Group Aa - Always Athletic, Always Eumenorrhoeic			
1	Cycling	100/200/400	Club vets
2	MDR/Hockey	LDR	County
3	Race-walking	Race-walking	International (MR)
4	Long jump	LDR	National/GB Vets
5	Hockey/squash	XC/Triathlon	National vets
6	MDR	LDR	World vets
7	LDR/racquet	LDR	Club
8	Hockey	Hockey	Club
9	400/800	400/800	World vets (R+M)
10	800/1500/XC	800/1500/XC	National vets (M)
11	400/800	400/200	National vets(M)
12	LDR/netball	XC/LDR	Club
13	Javelin	Javelin	World vets (M)
14	LDR	LDR	International(M)
15	LDR	LDR	Club vets
Group Ab - Always Athletic, Irregular Menstruation			
1	LDR/Aerobics	LDR/Swimmming	National vets
2	1500/3000m	LDR	World vets(M)
3	XC/LDR	LDR	National vets
4	3000/5000	LDR	World vets (M)
5	Keep Fit/Badmint	LDR	Club
6	Hockey/tennis/all	LDR/Cycling	County
7	Keep fit/squash	LDR	National vets
8	badminton	MDR	World vets

KEY

MDR - middle-distance running
LDR - long-distance running
XC - cross-country running
vets - veteran races
M - medal winner in class
R - record holder for age group

Premenopausal Veteran Athletes

Sporting Details of Late-Starter Athletes

Number of Athlete	Previous sport	Sport now	Level now
1		LDR	Club
2		LDR	World vets (M)
3		UD Walking	International (R)
4		LDR/Badminton	National vets
5		LDR	International
6		LDR	National vets
7		UD Walking	International (M)
8		Tri(R/S/Shoot)	National vets(M)
9		Triathlon/LDR	Club
10		LDR	Club
11		LDR	County
12		LDR	World vets(M)
13		LDR/Aerobics	Club
14		LDR	Club
15		LDR	World Vets (M)
16		MDR	Nat(M),Euro vets
17		LDR	International (M)
18		LDR	National
19		LDR	National vets
20		LDR	Club
21		LDR	Club
22		LDR/Swim	Club

Group Bb - Late-starter, Irregular Menstruation

1		LDR	National vets
2		LDR	National vets
3		LDR/swim	Club
4		LDR/Swim	Club
5		LDR	Club

KEY

MDR - middle-distance running
LDR - long-distance running
XC - cross-country running
UD - ultra-distance
vets - veteran races
M - medal winner in class
R - record holder for age group

Premenopausal Veteran Athletes

Bone Mineral Density of Neck of Femur of All-Timer Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	Z-score (SD)	T-score (SD)
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	0.766	0.828	-0.505	-1.290
2	0.849	0.935	0.236	-0.450
3	0.850	0.936	0.288	-0.450
4	0.876	0.970	0.477	-0.190
5	0.688	0.732	-1.288	-2.070
6	1.108	1.295	2.692	2.130
7	0.927	1.038	0.911	0.330
8	0.921	1.030	0.856	0.270
9	1.142	1.347	2.752	2.480
10	0.846	0.931	0.228	-0.490
11	0.778	0.843	-0.378	-1.170
12	1.023	1.172	1.842	1.290
13	1.060	1.225	2.069	1.650
14	0.855	0.942	0.547	-0.400
15	0.808	0.881	-0.026	-0.870
Group Ab - Always Athletic, Irregular Menstruation				
1	0.909	1.014	0.767	0.150
2	0.869	0.961	0.368	-0.250
3	0.903	1.006	0.732	0.080
4	0.896	0.997	0.624	0.020
5	0.836	0.918	0.108	-0.580
6	0.797	0.867	-0.265	-0.970
7	0.942	1.059	1.090	0.470
8	0.889	0.987	0.726	-0.060

Premenopausal Veteran Athletes

Bone Mineral Density of Neck of Femur of Late-Starter Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	Z-score (SD)	T-score (SD)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	0.885	0.982	0.688	-0.090
2	0.821	0.898	0.001	-0.740
3	1.002	1.142	1.641	1.070
4	0.862	0.952	0.426	-0.330
5	1.025	1.175	1.943	1.300
6	1.035	1.189	2.049	1.410
7	0.891	0.990	0.682	-0.040
8	0.974	1.103	1.481	0.790
9	0.880	0.975	0.536	-0.150
10	0.793	0.862	-0.285	-1.020
11	0.983	1.115	1.560	0.880
12	0.701	0.747	-1.306	-1.940
13	0.861	0.950	0.458	-0.330
14	0.943	1.060	1.372	0.490
15	0.958	1.081	1.318	0.640
16	0.913	1.019	0.993	0.190
17	0.959	1.082	1.201	0.640
18	0.865	0.955	0.413	-0.300
19	0.790	0.858	-0.295	-1.050
20	0.910	1.015	0.839	0.150
21	0.855	0.942	0.274	-0.400
22	0.910	1.015	0.881	0.160
Group Bb - Late-starter, Irregular Menstruation				
1	0.749	0.807	-0.646	-1.450
2	0.954	1.075	1.177	0.600
3	0.905	1.009	0.708	0.110
4	0.780	0.846	-0.441	-1.150
5	0.929	1.041	0.951	0.350

Premenopausal Veteran Athletes

Bone Mineral Density of Trochanteric Region in All-Timer Athletes

Number of Athlete	g.cm^{-2}	Specified Density	Z-score (SD)	T-score (SD)
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	0.664	0.703	-0.463	-0.650
2	0.820	0.897	1.167	1.090
3	0.684	0.727	-0.237	-0.420
4	0.782	0.848	0.795	0.670
5	0.754	0.813	0.511	0.360
6	0.958	1.081	2.412	2.620
7	0.767	0.829	0.644	0.500
8	0.758	0.818	0.552	0.400
9	0.912	1.018	2.013	2.110
10	0.746	0.803	0.429	0.270
11	0.801	0.872	0.983	0.880
12	0.812	0.887	1.090	1.000
13	0.931	1.044	2.180	2.333
14	0.684	0.727	-0.237	-0.420
15	0.998	1.136	2.747	3.070
Group Ab - Always Athletic, Irregular Menstruation				
1	0.776	0.841	0.735	0.600
2	0.732	0.786	0.283	0.110
3	0.775	0.839	0.725	0.590
4	0.763	0.824	0.603	0.460
5	0.763	0.824	0.603	0.450
6	0.587	0.612	-1.390	-1.490
7	0.728	0.781	0.240	0.060
8	0.800	0.871	0.973	0.870

Premenopausal Veteran Athletes

Bone Mineral Density of Trochanteric Region in Late-Starter Athletes

Number of Athlete	g.cm^{-2}	Specified Density	Z-score (SD)	T-score (SD)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	0.666	0.705	-0.440	-0.620
2	0.744	0.801	0.408	0.240
3	0.943	1.060	2.284	2.450
4	0.721	0.772	0.166	-0.010
5	0.839	0.921	1.347	1.300
6	0.837	0.919	1.329	1.270
7	0.695	0.740	-0.116	-0.300
8	0.790	0.858	0.874	0.750
9	0.796	0.866	0.934	0.830
10	0.716	0.766	0.112	-0.070
11	0.892	0.991	1.835	1.890
12	0.540	0.557	-2.010	-2.020
13	0.690	0.734	-0.171	-0.360
14	0.805	0.878	1.022	0.930
15	0.701	0.747	-0.050	-0.230
16	0.905	1.009	1.951	2.030
17	0.771	0.834	0.685	0.540
18	0.799	0.870	0.963	0.860
19	0.634	0.667	-0.813	-0.980
20	0.750	0.808	0.470	0.310
21	0.633	0.666	-0.825	-0.990
22	0.701	0.747	-0.050	-0.200
Group Bb - Late-starter, Irregular Menstruation				
1	0.701	0.747	-0.050	-0.230
2	0.794	0.864	0.914	0.800
3	0.731	0.784	0.272	0.100
4	0.588	0.613	-1.378	-1.490
5	0.800	0.871	0.973	0.860

Premenopausal Veteran Athletes

Bone Mineral Density of Lumbar Spine in All-Timer Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	Z-score (SD)	T-score (SD)
Group Aa - Always Athletic, Always Eumenorrhoeic				
1	1.135	1.336	0.873	0.510
2	1.145	1.351	0.962	0.600
3	0.960	1.084	-0.799	-1.080
4	1.153	1.363	1.034	0.670
5	1.075	1.247	0.321	-0.040
6	1.403	1.773	3.132	2.950
7	1.263	1.536	1.987	1.670
8	1.080	1.254	0.368	0.010
9	1.123	1.318	0.764	0.400
10	0.987	1.121	-0.527	-0.840
11	1.170	1.389	1.185	0.830
12	1.327	1.642	2.519	2.260
13	1.570	2.085	4.425	4.460
14	0.910	1.015	-1.317	-1.540
15	1.124	1.319	0.773	0.410
Group Ab - Always Athletic, Irregular Menstruation				
1	1.023	1.172	-0.174	-0.510
2	1.029	1.180	-0.116	-0.450
3	1.064	1.231	0.218	-0.140
4	1.021	1.169	-0.193	-0.530
5	1.027	1.177	-0.135	-0.470
6	0.906	1.010	-1.359	-1.570
7	1.039	1.195	-0.020	-0.360
8	1.088	1.266	0.443	0.080

Premenopausal Veteran Athletes

Bone Mineral Density of Lumbar Spine in Late-Starter Athletes

Number of Athlete	$g.cm^{-2}$	Specified Density	Z-score (SD)	T-score (SD)
Group Ba - Late-starter, Always Eumenorrhoeic				
1	0.975	1.104	-0.647	-0.950
2	1.039	1.195	-0.020	-0.360
3	1.157	1.369	1.070	0.710
4	1.083	1.259	0.396	0.040
5	1.140	1.343	0.918	0.550
6	1.051	1.212	0.095	-0.260
7	1.038	1.193	-0.029	-0.370
8	1.196	1.430	1.413	1.060
9	1.068	1.237	0.256	-0.100
10	1.035	1.189	-0.058	-0.400
11	1.358	1.695	2.771	2.540
12	1.056	1.219	0.142	-0.210
13	1.045	1.203	0.038	-0.310
14	1.230	1.483	1.707	1.380
15	1.083	1.259	0.396	0.040
16	1.114	1.304	0.682	0.320
17	1.009	1.152	-0.310	-0.640
18	0.997	1.135	-0.428	-0.750
19	0.979	1.110	-0.607	-0.910
20	1.145	1.351	0.962	0.600
21	1.010	1.153	-0.300	-0.620
22	1.125	1.321	0.782	0.420
Group Bb - Late-starter, Irregular Menstruation				
1	0.955	1.077	-0.850	-1.130
2	1.122	1.316	0.755	0.390
3	1.271	1.549	2.055	1.750
4	0.891	0.990	-1.520	-1.710
5	1.016	1.162	-0.242	-0.570

Premenopausal Veteran Athletes

Hormone Levels in All-Timer Athletes

Number of Athlete	Oestradiol ($pg.ml^{-1}$)	FSH ($mIU.l^{-1}$)
Group Aa - Always Athletic, Always Eumenorrhoeic		
1	258	20.3
2	350	7.8
3	1452	3.1
4	371	4.8
5	<70	24.4
6	604	4.2
7	395	2.4
8	334	3.9
9	319	59.7
10	632	6.8
11	150	21.1
12	700	11.9
13	212	64.4
14	990	6.1
15	399	3.1
Group Ab - Always Athletic, Irregular Menstruation		
1	271	2.8
2	221	22.6
3	458	7.1
4	747	4.3
5	737	49.1
6	<70	4.4
7	280	1.7
8	537	6.1

Premenopausal Veteran Athletes

Serum Hormone Levels in Late-Starter Athletes

Number of Athlete	Oestradiol ($pg.ml^{-1}$)	FSH ($mIU.l^{-1}$)
1	366	4.1
2	1004	5.3
3	336	3.3
4	160	62.7
5	<70	15.1
6	655	14.4
7	<70	0.5
8	405	5.9
9	870	17.9
10	78	58.5
11	1281	10.3
12	75	75.2
13	706	2.9
14	610	18.5
15	134	7
16	467	51
17	245	5.4
18	221	13
19	280	8.3
20	1014	5.7
21	175	25.5
22	2886	6.1

Group Bb - Late-starter, Irregular Menstruation

1	449	20.7
2	349	4.5
3	431	4.4
4	197	3.5
5	149	37.2

Table 1

Linear regression of BMD ($g.cm^{-2}$) with possible independent variables

Factor	Neck of Femur		Trochanteric		Lumbar Spine	
	F	p	F	p	F	p
Age	1.590	0.210	0.200	0.660	0.040	0.84
Menstrual status	0.759	0.388	1.743	0.193	**4.240**	**0.045**
Height	2.760	0.100	**6.150**	**0.017**	3.450	0.070
Weight	0.610	0.440	0.240	0.630	2.980	0.090
Body Mass Index	0.040	0.840	1.000	0.320	0.510	0.480
%fat	0.860	0.360	2.470	0.120	0.020	0.880
Fat free mass	3.120	0.080	2.590	0.110	2.210	0.140
Years of eumenorrhoea	0.200	0.660	1.140	0.290	3.210	0.080
No. Pregnancies	2.600	0.110	**4.000**	**0.051**	**5.240**	**0.030**
Mths breastfeeding	1.570	0.220	1.220	0.280	0.000	1.000
Grip strength (dominant)	0.040	0.850	**4.300**	**0.040**	**4.930**	**0.030**
Grip stremgth (non-dom)	0.090	0.770	2.000	0.160	1.890	0.180
Miles run per week	1.050	0.310	3.270	0.080	2.530	0.120
Hours trained per week	3.870	0.060	**6.480**	**0.014**	**6.710**	**0.013**
Dietary calcium intake	0.000	0.980	0.000	0.960	0.050	0.820
Peak Torque ext $60^0.sec^{-1}$	2.210	0.140	3.840	0.060	3.850	0.080
Peak Torque ext $180^0.sec^{-1}$	0.300	0.580	0.830	0.370	0.100	0.760
Peak Torque flex $60^0.sec^{-1}$	0.170	0.680	0.240	0.630	0.030	0.880
Peak Torque flex $180^0.sec^{-1}$	0.660	0.420	0.490	0.490	0.620	0.430
VO_2max	0.520	0.480	0.240	0.630	**6.650**	**0.013**
***HPW minus 2 outliers**			2.329	0.134	2.150	0.150

Figures shown in bold are those in which a trend towards significance is noted.

F: F ratio
p: pvalue

Table 2

Linear regression analysis of BMD (Z-score) with possible independent variables

Factor	Neck of Femur		Trochanteric		Lumbar Spine	
	F	p	F	p	F	p
Age	0.500	0.480	0.150	0.710	0.040	0.840
Menstrual status	1.060	0.310	1.570	0.220	**4.520**	**0.039**
Height	2.530	0.119	**5.980**	**0.018**	3.394	0.072
Weight	0.499	0.483	0.235	0.630	2.996	0.090
Body Mass Index	0.059	0.808	0.964	0.331	0.530	0.470
%fat	0.555	0.460	2.132	0.151	0.015	0.904
Years of eumenorrhoea	0.586	0.448	1.027	0.316	3.310	0.075
No. Pregnancies	2.475	0.122	**3.980**	**0.052**	**5.216**	**0.027**
Mths breastfeeding	1.503	0.226	1.273	0.265	0.006	0.937
Grip strength (dominant)	0.013	0.909	**3.902**	**0.054**	**4.708**	**0.035**
Grip stremgth (non-dom)	0.161	0.690	1.850	0.180	1.757	0.191
Miles run per week	1.199	0.279	3.293	0.076	2.488	0.121
Hours trained per week	3.568	0.065	**6.805**	**0.012**	**7.055**	**0.011**
VO_2max	0.730	0.397	0.276	0.602	**6.619**	**0.013**

Figures shown in bold are those in which a trend towards significance is noted.

F: F ratio
p: p value

Table 3

Multiple regression analysis of BMD (Z-score) of trochanteric region of proximal femur in premenopausal veteran athletes

Variable	Stage 1		Stage 2	
	F1,48	p-value	F1,46	p-value
Height	5.98	0.018	6.56	<0.05
Number of pregnancies	3.98	0.052	2.78	NS
Grip strength (dominant)	3.90	0.054	2.95	NS
Hours trained per week	6.82	0.012	6.30	<0.05
Menstrual status	1.57	0.22		

Menstrual status added in at stage 2

Table 4

Multiple regression analysis of BMD (Z-score) of lumbar spine (L2-L4) in premenopausal veteran athletes

Variable	Stage 1		Stage 2	
	F1,48	**p-value**	**F1,46**	**p-value**
Height	3.39	0.0725	4.26	0.05
Number of pregnancies	5.22	0.027	5.33	<0.05
Grip strength (dominant)	4.71	0.035	3.08	NS
Hours trained per week	7.06	0.011	6.52	<0.05
VO_2max ($ml.kg^{-1}.min^{-1}$)	6.62	0.013	5.55	<0.05
Peak Torque (ext. 60^0sec^{-1})	3.85	0.056	5.10	<0.05
Menstrual status	4.52	0.039		

Menstrual status added in at stage 2

APPENDIX 3

DATA FOR POSTMENOPAUSAL VETERAN ATHLETES

Postmenopausal Athletes

Demographic Variables

Number of Athlete	Age (years)	Height (cm)	Weight (kg)	Body Mass Index ($kg.m^{-2}$)
All-Timer Athletes				
1	58	161.0	58.5	22.57
2	55	164.0	57.0	21.19
3	49	170.0	56.0	19.38
4	58	173.0	74.0	24.73
5	51	170.0	65.5	22.66
6	51	163.5	53.9	20.16
7	61	161.0	56.0	21.60
8	49	165.9	55.9	20.31
9	55	182.0	75.5	22.79
10	59	161.0	56.3	21.72
Late-Starter Athletes				
1	56	167.6	52.3	18.60
2	57	165.2	65.5	24.00
3	54	162.0	64.3	24.50
4	62	158.3	58.7	23.42
5	50	163.8	58.6	21.84
6	51	161.0	61.3	23.63
7	63	159.0	43.0	17.01
8	50	161.7	63.2	24.17
9	68	151.3	53.6	23.41
10	65	163.4	58.5	21.91
11	56	155.7	49.0	20.21
12	48	157.8	54.5	21.89
13	52	166.2	69.5	25.16
14	59	158.2	55.3	22.10

Postmenopausal Athletes

Body Composition Details

Number of Athlete	Body Fat 1 (%)	Total Body Potassium (mmol)	Fat Free Mass (kg)	Body Fat (kg)	Body Fat 2 (%)	Fat Free Mass (%)
All-Timer Athletes						
1	29.2	2748.0	45.80	12.7	21.7	78.3
2	28.0	2484.0	41.40	14.6	25.6	72.6
3	19.9	3048.0	50.80	5.2	9.3	90.7
4	34.1	3408.0	56.80	17.2	23.2	76.8
5	30.9	2910.0	48.50	17.0	26.0	74.0
6	26.5	2245.5	37.40	16.5	30.6	69.4
7	32.7	2298.0	38.30	17.7	31.6	68.4
8	23.6	2852.4	47.54	8.4	15.0	85.0
9	28.0	3426.0	57.10	18.4	24.4	75.6
10	29.6	2334.0	38.90	17.1	30.4	69.1
Late-Starter Athletes						
1	22.7	2760.0	46.00	6.3	12.0	88.0
2	34.1	2369.5	39.49	26.0	39.7	60.3
3	33.0	2429.7	40.50	23.8	37.0	63.0
4	34.0	2400.0	40.00	18.7	31.9	68.1
5	29.4	2354.9	39.25	19.4	33.0	67.0
6	32.0	*	*	*	*	0.0
7	16.9	2142.0	35.70	7.3	17.0	83.0
8	31.3	2895.9	48.27	14.9	23.6	76.4
9	31.6	2190.0	36.50	17.1	31.9	68.1
10	27.5	2946.0	49.10	9.4	16.1	83.9
11	18.9	2703.6	45.10	3.9	8.0	92.0
12	23.6	2916.0	48.60	5.9	10.8	89.2
13	35.1	2832.0	47.20	22.3	32.1	67.9
14	29.2	2099.3	34.99	20.3	36.7	63.3

* data not available

Postmenopausal Athletes

Gynaecological History

Number of Athlete	Age at Menarche (years)	Years of EU	Years of OL/AM	Years PM
All-Timer Athletes				
1	15.0	32.0	11.0	11.0
2	11.0	37.0	5.0	5.0
3	12.0	34.0	2.0	2.0
4	13.8	35.0	10.5	8.0
5	12.8	35.0	4.0	2.0
6	13.0	*	*	1.0
7	14.8	31.0	15.0	15.0
8	12.0	32.0	5.5	1.5
9	15.0	35.0	7.0	3.0
10	15.0	34.0	23.0	12.0
Late-Starter Athletes				
1	16.3	38.0	6.0	7.0
2	11.0	31.0	10.0	7.0
3	15.0	37.0	1.0	3.0
4	16.0	37.0	9.0	7.0
5	13.0	29.0	8.0	5.0
6	13.0	37.0	0.0	1.0
7	17.0	32.0	14.0	14.0
8	14.2	36.0	0.0	0.0
9	13.0	39.0	18.0	14.0
10	15.0	45.0	5.0	9.0
11	16.0	34.0	1.0	5.0
12	11.5	33.0	4.5	1.5
13	13.0	37.0	2.0	2.0
14	12.5	28.0	17.0	16.0

KEY

*data not available
OL/AM: oligomenorrhoea or amenorrhoea
EU: eumenorrhoea
PM: postmenopause

Postmenopausal Athletes

Obstetric History

Number of Athlete	OCP use (years)	Parity	Breast fed (months)	HRT use (years)
All-Timer Athletes				
1	9.0	2	14.0	0.0
2	0.0	3	1.0	0.0
3	0.0	4	16.0	0.0
4	0.0	1	6.0	0.0
5	0.0	0	0.0	0.0
6	1.6	3	4.0	0.0
7	0.0	1	1.5	5.0
8	15.0	0	0.0	0.0
9	0.0	0	0.0	1.5
10	0.0	3	0.0	4.0
Late-Starter Athletes				
1	0.0	2	9.0	1.0
2	0.0	1	1.5	0.0
3	0.0	4	17.0	2.0
4	0.0	5	10.0	0.0
5	0.5	3	9.0	0.5
6	17.0	2	3.0	0.0
7	10.0	2	0.3	0.0
8	4.5	3	0.0	0.0
9	0.0	4	24.0	0.0
10	0.0	0	0.0	6.0
11	2.0	2	14.0	0.0
12	5.0	2	10.0	0.0
13	0.0	0	0.0	0.0
14	2.0	2	0.0	2.0

KEY
OCP: oral contraceptive
HRT: hormone replacement therapy

Postmenopausal Athletes

Training Details

Number of Athlete	Distance Run Per Week (miles)	Time Trained Per Week (hours)	Sessions per week	Age Started	Years of Exercise
All-Timer Athletes					
1	24	8	4		40
2	50	9	10		37
3	32	5	5		31
4	N/A	10	5		40
5	30	4	8		33
6	24	5	5		33
7	70	11	11		43
8	40	10	7		31
9	35	6	4		37
10	20	5	5		41
Late-Starter Athletes					
1	20	3	3	48	8
2	40	6	5	49	8
3	25	6	4	50	4
4	30	5	6	53	9
5	20	8	5	35	15
6	12	5	5	45	6
7	30	6	6	54	9
8	18	10	6	46	4
9	15	7	6	60	8
10	25	4	5	54	9
11	45	10	7	51	5
12	30	9	6	44	4
13	20	3	3	44	8
14	20	4	4	51	9

Postmenopausal Athletes

Maximal Oxygen Uptake and Grip Strength

Number of Athlete	VO2max ($ml.kg^{-1}.min^{-1}$)	Grip Strength Dominant Hand (Kgf)	Grip Strength Non-Dominant Hand (Kgf)
All-Timer Athletes			
1	39.8	30.5	28.5
2	45.5	33.5	28.5
3	49.6	37.0	38.5
4	30.0	50.5	46.0
5	43.1	38.5	29.5
6	45.3	29.5	25.0
7	40.2	27.5	25.5
8	53.8	35.0	33.5
9	40.6	40.0	32.0
10	injured	30.5	30.0
Late-Starter Athletes			
1	41.0	36.0	30.0
2	23.0	32.0	28.5
3	39.8	42.0	37.5
4	40.9	28.0	31.5
5	52.8	28.5	24.0
6	42.6	32.5	30.0
7	43.3	26.0	22.5
8	31.6	36.0	32.0
9	23.1	30.0	27.5
10	37.2	28.0	30.0
11	63.0	35.0	28.5
12	44.9	32.0	27.0
13	35.6	34.0	29.0
14	32.7	23.5	24.0

Postmenopausal Athletes

Dietary Calcium Intake and Serum Parameters of Bone Metabolism

Number of Athlete	Calcium Intake ($mg.day^{-1}$)	Serum Calcium ($mmol.l^{-1}$)	Serum Phosphate ($mmol.l^{-1}$)	Alkaline Phosphatase ($mmol.l^{-1}$)
All-Timer Athletes				
1	828	2.30	1.12	88.0
2	1629	2.48	1.64	87.0
3	932	2.35	1.19	77.0
4	1200	2.27	1.17	76.0
5	892	2.37	1.15	76.0
6	563	2.24	1.11	68.0
7	1090	2.26	1.33	45.0
8	457	2.27	1.39	74.0
9	1237	2.24	1.50	75.0
10	1033	*	*	*
Late-Starter Athletes				
1	973	2.27	0.97	50.0
2	872	2.32	1.41	124.0
3	885	2.28	1.20	64.0
4	1172	2.33	0.95	51.0
5	1013	2.43	1.36	86.0
6	922	2.32	1.24	63.0
7	1126	2.30	1.13	85.0
8	1038	2.26	1.34	82.0
9	1387	2.45	1.22	70.0
10	1222	2.31	1.42	104.0
11	1408	2.35	1.43	100.0
12	1037	2.33	1.39	88.0
13	877	2.23	1.60	81.0
14	1570	2.38	1.43	77.0

* data not available

Postmenopausal Athletes

Back Strength

Number of Athlete	Peak Torque Extension ($60^{o}.sec^{-1}$)	($180^{o}.sec^{-1}$)	Peak Torque Flexion ($60^{o}.sec^{-1}$)	($180^{o}.sec^{-1}$)
All-Timer Athletes				
1	144	114	155	152
2	217	209	148	125
3	179	195	155	188
4	266	199	249	336
5	245	277	232	193
6	92	62	73	56
7	146	115	126	89
8	186	155	133	111
9	244	224	172	157
10	167	144	140	161
Late-Starter Athletes				
1	122	171	144	99
2	174	125	164	110
3	108	68	137	73
4	157	129	146	75
5	133	91	117	87
6	149	118	115	76
7	155	144	179	179
8	167	148	99	76
9	161	114	92	77
10	111	103	122	68
11	83	152	62	85
12	198	178	114	96
13	225	244	149	161
14	172	163	107	85

Postmenopausal Athletes

Urinary Parameters of Bone Metabolism

Number of Athlete	Urinary Creatinine ($mmol.l^{-1}$)	Urinary Calcium ($mmol.l^{-1}$)	Urinary OHPR	Ca:creat ratio	OHPR:creat ratio
All-Timer Athletes					
1	7.0	2.8	133.00	0.40	19.00
2	7.3	2.9	150.00	0.40	20.55
3	12.2	3.6	229.00	0.30	18.77
4	5.8	1.4	103.00	0.24	17.76
5	7.6	2.6	131.00	0.34	17.24
6	5.2	3.2	125.00	0.62	24.04
7	6.0	1.2	150.00	0.20	25.00
8	8.5	4.2	120.00	0.49	14.12
9	14.0	6.1	45.00	0.44	3.21
10	6.9	1.5	28.00	0.22	4.06
Late-Starter Athletes					
1	11.0	2.3	145.00	0.21	13.18
2	5.5	3.0	166.00	0.55	30.18
3	8.0	5.0	97.00	0.63	12.13
4	15.9	10.3	138.00	0.65	8.68
5	7.5	5.0	163.00	0.67	21.73
6	19.3	5.7	316.00	0.30	16.37
7	8.2	5.5	215.00	0.67	26.22
8	5.1	0.9	115.00	0.18	22.55
9	5.5	1.2	98.00	0.22	17.82
10	5.6	3.2	111.00	0.57	19.82
11	4.3	2.9	131.00	0.67	30.47
12	10.5	7.3	86.00	0.70	8.19
13	19.7	3.1	49.00	0.16	2.49
14	5.6	4.5	168.00	0.80	30.00

KEY

Ca: calcium OHPR: hydroxyproline

Creat: creatinine

Postmenopausal Athletes

Sporting Details

Number of Athlete	Previous sport	Sport now	Level now
All-Timer Athletes			
1	100/200/400	LDR	World vets (6th 10k)
2	sprint hurdles	LDR	National vets
3	200/400/800	LD/MDR	World vets (M)
4	discus/pent	golf/keep fit/athletics	World vets (MR)
5	Racquet sports	LDR	World vets (M)
6	Racquet sports	LDR/golf	Club vets
7	MDR	LDR	World vets (M+R)
8	800/1 mile	800/LDR	National
9	Rowing	LD walking	Intl (MR)
10	100 - 800m	LDR	National vets
Late-Starter Athletes			
1		LDR	World vets (M)
2		LDR	Intl. vets
3		LDR	Club vets
4		LDR	National vets(M)
5		MDR/XC	National vets
6		Tri (Run/Swim/Shoot)	National vets(M)
7		LDR	World vets (MR)
8		LDR	Club
9		LDR	World vets(M)
10		LDR	World vets(M)
11		LDR	World vets (M)
12		LDR	Natl vets
13		LDR	Club vets
14		LDR/Golf	Inter-island vets

KEY

LDR	Long-distance running
MDR	Middle-distance running
M	Medal winner at National or World Championships
R	World record holder for that age group

Postmenopausal Athletes

Bone Mineral Density of Neck of Femur

Number of Athlete	$g.cm^{-2}$	Specified Density	Z-score (SD)	Tscore (SD)
All-Timer Athletes				
1	0.729	0.782	-0.060	-1.650
2	0.862	0.952	0.882	-0.330
3	0.793	0.862	0.088	-1.020
4	0.811	0.885	0.578	-0.840
5	0.704	0.751	-0.800	-1.910
6	0.705	0.752	-0.843	-1.890
7	0.768	0.831	0.543	-1.270
8	0.786	0.853	-0.006	-1.080
9	0.783	0.850	0.046	-1.120
10	0.648	0.683	-0.872	-2.460
Late-Starter Athletes				
1	0.694	0.739	-0.638	-2.010
2	0.822	0.899	0.627	-0.730
3	1.077	1.250	2.529	1.830
4	0.809	0.883	0.506	-0.860
5	0.707	0.755	-0.608	-1.870
6	0.822	0.899	0.306	-0.730
7	0.553	0.572	-1.908	-3.420
8	0.811	0.885	0.204	-0.840
9	0.653	0.689	-0.709	-2.410
10	0.831	0.911	0.817	-0.630
11	0.662	0.700	-1.091	-2.330
12	0.859	0.948	0.668	-0.360
13	0.813	0.888	0.276	-0.810
14	0.851	0.937	1.373	-0.440

KEY

SD: standard deviations

Postmenopausal Athletes

Bone Mineral Density of Trochanteric Region

Number of Athlete	g.cm^{-2}	Specified Density	Z-score (SD)	Tscore (SD)
All-Timer Athletes				
1	0.638	0.672	-0.188	-0.930
2	0.635	0.668	-0.472	-0.970
3	0.793	0.862	1.057	0.790
4	0.743	0.799	0.774	0.230
5	0.718	0.768	0.257	-0.040
6	0.678	0.719	-0.202	-0.490
7	0.601	0.628	-0.391	-1.340
8	0.659	0.697	-0.376	-0.700
9	0.655	0.692	-0.353	-0.750
10	0.628	0.660	-0.247	-1.050
Late-Starter Athletes				
1	0.598	0.624	-0.759	-1.380
2	0.767	0.829	0.988	0.500
3	0.822	0.899	1.416	1.110
4	0.596	0.622	-0.779	-1.400
5	0.572	0.594	-1.101	-1.660
6	0.808	0.881	1.178	0.960
7	0.426	0.430	-2.118	-3.290
8	0.692	0.736	-0.057	-0.330
9	0.499	0.511	-1.430	-2.480
10	0.594	0.620	-0.714	-1.420
11	0.589	0.614	-0.933	-1.480
12	0.692	0.736	-0.036	-0.330
13	0.660	0.698	-0.344	-0.690
14	0.538	0.555	-0.969	-2.050

KEY

SD: standard deviations

Postmenopausal Athletes

Bone Mineral Density of Lumbar Spine

Number of Athlete	g.cm^{-2}	Specified Density	Z-score (SD)	Tscore (SD)
All-Timer Athletes				
1	0.888	0.986	-0.090	-1.730
2	1.032	1.185	0.847	-0.420
3	1.032	1.185	0.757	-0.420
4	0.978	1.108	0.505	-0.920
5	1.105	1.291	1.390	0.240
6	1.111	1.300	1.427	0.290
7	0.839	0.921	-0.290	-1.990
8	0.922	1.032	-0.293	-1.430
9	0.856	0.944	-0.841	-2.030
10	0.824	0.902	-0.558	-2.320
Late-Starter Athletes				
1	0.981	1.113	0.494	-0.890
2	0.986	1.120	0.535	-0.850
3	0.895	0.995	-0.460	-1.670
4	0.870	0.962	-0.445	-1.900
5	0.914	1.021	-0.165	-1.500
6	1.026	1.176	0.668	-0.480
7	0.783	0.850	-0.785	-2.690
8	1.021	1.169	0.622	-0.530
9	0.783	0.850	-0.785	-2.690
10	0.840	0.923	-0.592	-2.170
11	0.769	0.832	-1.556	-2.820
12	0.909	1.014	-0.421	-1.540
13	1.000	1.139	0.469	-0.720
14	0.965	1.090	0.645	-1.040

KEY

SD: standard deviations

Postmenopausal Athletes

Serum Hormone Levels

Number of Athlete	Oestradiol ($pgl.ml^{-1}$)	FSH ($mIU.l^{-1}$)
All-Timer Athletes		
1	<70	76.6
2	<70	94.8
3	<70	73.8
4	<70	78.4
5	99	64.1
6	195	33.6
7	<70	43.2
8	<70	76.2
9	89	44.8
10	91	51
Late-Starter Athletes		
1	<70	36.5
2	<70	80.7
3	<70	50.9
4	<70	63.5
5	70	57.3
6	180	65.4
7	<70	<0.2
8	80	87.9
9	<70	82.6
10	<70	90.7
11	<70	61.4
12	75	76.5
13	<70	80.9
14	<70	80.5

APPENDIX 4

DATA AND FURTHER STATISTICAL ANALYSIS FOR TREATMENT INTERVENTION STUDY

In this appendix the following key applies:

1 1st visit

2 2nd visit (9 month follow-up)

Treatment Intervention Study

Gynaecological History

Number of Athlete	Age	Number of menses year before study	Years of AM/OL	Menstruation During Study	Follow-up (months)
Treatment with HRT and Calcium					
1	27	0	10	EU	9
2	28	0	7.5	EU	9
3	34	0	12	EU	9
4	29	0	7	EU	9
5	32	0	3	EU	11
6	19	4	3	EU	10
7	30	0	6	EU	8
Treatment with Calcium					
1	27	9	1.5	EU	11
2	31	0	11	AM	25
3	18	0	1.5	AM	12
4	30	0	8	EU	9
5	32	0	4.5	OL	9
6	27	0	12	AM	9
7	34	0	11	OL	9
8	35	6	8	EU	9
9	34	4	9	EU	9
10	19	0	5	AM	10
Controls					
1	35	0	17	AM	11
2	21	0	5	AM	16
3	18	6	1.5	EU	14
4	27	6	12	EU	9
5	25	0	4	OL	9
6	29	4	3	EU	10
7	22	0	6	AM	9
8	28	0	5	AM	9
9	20	0	7	AM	18

KEY

AM: amenorrhoeic (0-3 cycles per year)

EU: eumenorrhoeic (10^{-1}3 cycles per year)

OL: oligomenorrhoeic (4-9 cycles per year)

Treatment Intervention Study

Treatment compliance and side effects

No. of Athlete	SE's	Compliance	Comments
Treatment with HRT and Calcium			
1	yes	Ca+HRT-90%	Stopped HRT after 9 months.
2	yes	Ca40%HRT30%	Stopped HRT after 3 months. Remained EU
3	no	Ca+HRT>90%	Satisfied with treatment
4	yes	Ca+HRT>90%	Increased breast size and body weight
5	yes	Ca+HRT>90%	Tender breasts.Stopped HRT at 10 mths, then EU
6	no	Ca+HRT>90%	Pelvic stress fractures prevented running
7	yes	Ca+HRT>90%	Chronic fatigue attributed to HRT.Constipation ?Ca. Stopped treatment after 8 mths
Treatment with Calcium			
1	no	Ca -80%	
2	yes	Ca-0%	Severe diarrhoea/abdominal pain with Calcium.
3	no	Ca-80%	
4	no	Ca-90%	
5	no	Ca-75%	
6	no	Ca->90%	
7	yes	Ca -0%	Calcium caused diarrhoea
8	yes	Ca>90%	
9	no	Ca>90%	
10	no	Ca>90%	
Controls			
1	N/A		
2	N/A		
3	N/A		
4	N/A		
5	N/A		**KEY**
6	N/A		HRT: hormone replacement therapy
7	N/A		Ca: calcium
8	N/A		SE's: Side effects
9	N/A		

Treatment Intervention Study

Morphological variables

No. of Athlete	Height (cm)	Weight (kg)	Weight (kg)	BMI ($kg.m^{-2}$)	BMI ($kg.m^{-2}$)	Body Fat (%)	Body Fat (%)
		1	2	1	2	1	2
Treatment with HRT and Calcium							
1	165	52.0	52.7	19.1	19.4	17.6	16.6
2	159	50.6	50.0	20.0	19.8	21.5	21.9
3	166	54.0	53.8	19.6	19.5	20.2	18.8
4	156	58.0	57.8	23.8	23.8	25.2	22.7
5	162	52.3	55.0	19.9	21.0	20.6	21.8
6	161	45.3	46.6	17.5	18.0	15.4	16.9
7	175	63.0	63.1	20.6	20.6	20.6	21.9
Treatment with Calcium							
1	169	57.0	56.4	20.0	19.7	25.7	24.8
2	169	53.9	52.9	18.9	18.5	21.1	17.8
3	166.5	48.7	48.7	17.6	17.5	12.6	10.2
4	163	48.0	51.6	18.1	19.4	17.9	16.4
5	156	51.5	51.6	21.2	21.2	24.7	23.1
6	164	37.6	37.5	14.0	13.9	7.1	6.2
7	165	52.0	53.0	19.1	19.5	15.9	17.8
8	166.5	57.5	59.5	20.7	21.5	25.1	24.4
9	166	56.0	57.0	20.3	20.7	23.2	23.7
10	164.5	48.7	48.9	18.0	18.1	16.8	17.4
Controls							
1	165	50.0	52.5	18.4	19.3	15.2	16.8
2	166.5	44.5	45.1	16.1	16.3	11.3	12.8
3	163	63.5	N/A	23.9	N/A	N/A	N/A
4	163	48.5	47.8	18.3	18.0	18.6	16.6
5	160	52.7	50.8	20.6	19.8	24.5	21.7
6	156	50.2	50.5	20.6	20.8	21.7	19.9
7	172	59.2	59.9	20.0	20.2	15.6	16.3
8	156	46.0	46.2	18.9	19.0	13.8	15.5

Treatment Intervention Study

Bone mineral density of Wards Triangle

Number of Athlete	BMD ($g.cm^{-2}$) 1	BMD ($g.cm^{-2}$) 2	Change per Year ($g.cm^{-2}$)	Change per Year (%)
Treatment with HRT and Calcium				
1	0.564	0.598	0.05	8.04
2	0.711	0.744	0.04	6.19
3	0.567	0.593	0.03	6.11
4	0.875	0.886	0.01	1.68
5	0.544	0.574	0.03	6.02
6	0.806	0.802	0.00	-0.60
7	0.863	0.848	-0.02	-2.61
Treatment with Calcium				
1	0.622	0.684	0.07	10.87
2	0.701	0.683	-0.01	-1.23
3	0.719	0.667	-0.05	-7.23
4	0.548	0.542	-0.01	-1.46
5	0.589	0.623	0.05	7.70
6	0.623	0.582	-0.05	-8.77
7	0.696	0.709	0.02	2.49
8	0.602	0.586	-0.02	-3.54
9	0.611	0.702	0.12	19.86
10	0.849	0.814	-0.04	-4.95
Controls				
1	0.726	0.708	-0.02	-2.70
2	0.736	0.649	-0.07	-8.87
3	0.919	0.933	0.01	1.31
4	1.006	0.996	-0.01	-1.33
5	0.608	0.624	0.02	3.51
6	0.763	0.78	0.02	2.67
7	0.758	0.744	-0.02	-2.46
8	0.7	0.678	-0.03	-4.19

Treatment Intervention Study

Bone mineral density of Neck of Femur

Number of Athlete	BMD ($g.cm^{-2}$) 1	BMD ($g.cm^{-2}$) 2	Change per Year ($g.cm^{-2}$)	Change per Year (%)
Treatment with HRT and Calcium				
1	0.763	0.77	0.009	1.223
2	0.796	0.77	-0.035	-4.355
3	0.772	0.762	-0.013	-1.727
4	0.962	0.952	-0.013	-1.386
5	0.711	0.726	0.016	2.301
6	0.816	0.82	0.005	0.588
7	0.968	0.961	-0.011	-1.085
Treatment with Calcium				
1	0.794	0.782	-0.013	-1.649
2	0.871	0.827	-0.021	-2.425
3	0.814	0.759	-0.055	-6.757
4	0.706	0.703	-0.004	-0.567
5	0.742	0.787	0.060	8.086
6	0.744	0.718	-0.035	-4.659
7	0.802	0.846	0.059	7.315
8	0.819	0.833	0.019	2.279
9	0.672	0.738	0.088	13.095
10	0.856	0.846	-0.012	-1.402
Controls				
1	0.808	0.791	-0.019	-2.295
2	0.801	0.721	-0.060	-7.491
3	0.816	0.834	0.015	1.891
4	1.099	1.114	0.020	1.820
5	0.77	0.781	0.015	1.905
6	0.803	0.853	0.060	7.472
7	0.837	0.821	-0.021	-2.549
8	0.769	0.76	-0.012	-1.560
9	0.913	0.921	0.005	0.584

Treatment Intervention Study

Bone mineral density of Trochanteric Region

Number of Athlete	BMD ($g.cm^{-2}$) 1	BMD ($g.cm^{-2}$) 2	Change per Year ($g.cm^{-2}$)	Change per Year (%)
Treatment with HRT and Calcium				
1	0.697	0.727	0.040	5.739
2	0.620	0.637	0.023	3.656
3	0.523	0.531	0.011	2.040
4	0.886	0.885	-0.001	-0.150
5	0.580	0.593	0.014	2.445
6	0.765	0.744	-0.025	-3.294
7	0.774	0.782	0.012	1.550
Treatment with Calcium				
1	0.665	0.667	0.002	0.328
2	0.601	0.606	0.002	0.399
3	0.782	0.729	-0.053	-6.777
4	0.541	0.569	0.037	6.901
5	0.506	0.530	0.032	6.324
6	0.625	0.582	-0.057	-9.173
7	0.704	0.720	0.021	3.030
8	0.709	0.696	-0.017	-2.445
9	0.681	0.688	0.009	1.371
10	0.861	0.838	-0.028	-3.206
Controls				
1	0.636	0.593	-0.047	-7.376
2	0.678	0.537	-0.106	-15.597
3	0.728	0.752	0.021	2.826
4	0.856	0.863	0.009	1.090
5	0.581	0.607	0.035	5.967
6	0.737	0.729	-0.010	-1.303
7	0.695	0.696	0.001	0.192
8	0.607	0.602	-0.007	-1.098
9	0.769	0.749	-0.013	-1.734

Treatment Intervention Study

Bone mineral density of Lumbar Spine (L2-L4)

Number of Athlete	BMD ($g.cm^{-2}$) 1	BMD ($g.cm^{-2}$) 2	Change per Year ($g.cm^{-2}$)	Change per Year (%)
Treatment with HRT and Calcium				
1	0.857	0.884	0.036	4.201
2	0.891	0.902	0.015	1.646
3	0.713	0.850	0.183	25.619
4	1.231	1.209	-0.029	-2.383
5	0.878	0.945	0.073	8.325
6	0.961	0.953	-0.010	-0.999
7	1.054	1.077	0.034	3.273
Treatment with Calcium				
1	0.999	1.038	0.043	4.259
2	1.014	0.953	-0.029	-2.888
3	1.013	0.902	-0.111	-10.958
4	0.860	0.887	0.036	4.186
5	0.825	0.834	0.011	1.389
6	0.762	0.747	-0.020	-2.625
7	0.991	1.023	0.043	4.305
8	1.040	1.025	-0.020	-1.923
9	1.044	1.086	0.056	5.364
10	1.104	1.091	-0.016	-1.413
Controls				
1	0.937	0.923	-0.015	-1.630
2	0.877	0.745	-0.099	-11.288
3	0.867	0.907	0.034	3.955
4	1.088	1.086	-0.003	-0.245
5	0.770	0.790	0.027	3.463
6	1.009	1.028	0.023	2.260
7	1.002	0.991	-0.015	-1.464
8	0.856	0.872	0.021	2.492
9	0.954	0.952	-0.001	-0.140

Treatment Intervention Study

Distance run per week and maximal aerobic capacity (VO2max)

Number of Athlete	Distance run per week 1	Distance run per week 2	VO2max ($ml.kg^{-1}.min^{-1}$) 1	VO2max ($ml.kg^{-1}.min^{-1}$) 2
Treatment with HRT and Calcium				
1	32	19	59.6	52.6
2	28	25	43.9	45.3
3	30	35	54.4	59.8
4	30	20	57.8	59.7
5	80	70	61.1	54.1
6	50	45	69.0	61.2
7	65	50	53.5	57.5
Treatment with Calcium				
1	25	*	57.2	injured
2	85	60	63.1	unwell
3	50	60	injured	injured
4	35	25	39.9	injured
5	40	40	55.5	50.5
6	90	90	60.7	54.5
7	80	65	59.4	56.2
8	55	65	50.2	47.8
9	40	40	51.7	55.6
10	50	50	66.3	65.5
Controls				
1	70	25	58.3	45.63
2	60	60	70.8	injured
3	50	*	61.6	injured
4	38	26	56.3	58.1
5	45	60	59.0	64.1
6	70	50	65.4	64.3
7	30	25	57.6	62.8
8	55	55	61.0	injured
9	60	45	65.9	injured

* athlete currently resting due to injury/illness

Treatment Intervention Study

Dietary Calcium Intake and Serum Parameters of Bone Metabolism

No. of Athlete	Calcium Intake ($mg.day^{-1}$)		Serum Calcium ($mmol.l^{-1}$)		Serum Phosphate ($mmol.l^{-1}$)		Alkaline Phosphatase ($mmol.l^{-1}$)	
	1	2	1	2	1	2	1	2
Treatment with HRT and Calcium								
1	1215	2215	2.37	2.28	1.45	1.26	54	46
2	1935	1400	2.23	2.25	1.42	1.41	53	57
3	327	1327	2.35	2.3	1.17	1.08	77	51
4	766	1766	2.38	2.44	0.99	1.1	40	47
5	962	1962	2.33	2.32	1.03	1.23	85	83
6	1066	2066	2.37	2.35	1.11	1.21	63	61
7	630	1974	2.38	2.35	1.11	1	54	50
Treatment with Calcium								
1	476	2205	2.23	2.28	0.9	1.43	63	71
2	1118	1903	2.37	2.29	1.4	1.27	67	75
3	1134	1874	2.21	2.19	1.28	1.25	43	55
4	587	1587	2.25	2.33	1.18	1.31	109	84
5	398	1398	2.39	2.3	1.1	1.19	69	45
6	933	1933	2.45	2.3	1.24	1.16	46	41
7	981	981	2.21	2.21	1.13	1.13	49	49
8	583	1583	2.29	2.4	1.19	1.32	37	46
9	1002	2000	2.31	2.25	1.02	1.28	47	40
10	942	1942	2.2	2.41	1.42	1.3	61	57
Controls								
1	1009	1009	2.28	2.22	1.24	1.34	58	45
2	1355	886	2.45	2.18	1.23	1.01	35	48
3	1063							
4	590	590	2.36	2.29	0.86	1.38	59	67
5	768	768	2.32	2.14	1.35	1.32	70	61
6	1814	1198	2.29	2.2	1.19	1.24	49	46
7	706	706	2.4	2.21	1.26	1.11	55	62
8	877	1254	2.33	2.22	1.3	1.49	63	53
9	1157	706	2.34	2.28	1.19	1.21	71	63

Treatment Intervention Study

Serum Hormone levels

Number of Athlete	Oestrogen ($pg.ml^{-1}$)		FSH ($mIU.l^{-1}$)		PRL ($mU.l^{-1}$)
	1	2	1	2	1
Treatment with HRT and Calcium					
1	157	1474	7.1	<0.2	218
2	157	861	0.5	7.8	134
3	174	858	10.3	7.7	185
4	161	1579	7.1	2.6	127
5	737	678	5.9	3.1	158
6	140	95	6	6	156
7	75	125	4.5	5.5	94
Treatment with Calcium					
1	820	179	5.2	6.6	125
2	143	N/A	8.7	N/A	134
3	75	77	3.2	5.2	127
4	70	104	5.5	4.8	113
5	134	70	9.1	0.3	106
6	77	93	0.8	0.5	394
7	70	110	4.8	5.2	146
8	80	222	8.5	6.2	82
9	76	544	2.6	2.5	350
10	90	98	5.4	8.8	314
Controls					
1	70	80	3	4	94
2	70	70	1.6	N/A	209
3	70	N/A	1.1	N/A	115
4	763	N/A	8.7	N/A	N/A
5	169	102	7.5	7	161
6	368	331	3.2	6.6	142
7	127	184	8.1	5.2	173
8	165	74	4.4	4.5	166
9	137	N/A	7.8	N/A	187

Power calculations for a full study of the effectiveness of HRT in amenorrhoeic athletes using Lumbar Spine BMD as the end-point

Calculation 1

Let us assume a true effect of 5% per year at the spine.

SD of an individual change = 6.37% of initial value per year (from Table 3c, Chapter 8)

If no dropouts and full attendance;

$$n > 2\,((u_{2\alpha} + u_{2\beta})\,\sigma / \delta_o)^2$$

where $\delta_o = 5\%$, $\sigma = 6.37$, n = number in each group

$u_{2\alpha}$ defines the sgnificance and $u_{2\beta}$ the power. For a power of 80% and significance of 5% they are 1.96 and 0.84.

Therefore n = 25

Calculation 2

However 30% 'converted' one way or another to the alternative group:

Group			**Density Change**	**Average Change**
Calcium/ non-treatment		70%	no change	
Regained menses		30%	+5%	1.5%
HRT	Continued	60%	+5%	
HRT	Withdrew	40%	no change	3.0%

Mean difference = + 1.5% = δ_o so n > 280

However, non-attenders = 50% and n must be doubled to 560 in each group

Calculation 3

Power calculation using amenorrhoeic athletes only:

Group			**Density Change**	**Average Change**
Calcium/ placebo		92%	no change	
Regained menses		8%	+5%	0.4%
HRT	Continued	60%	+5%	
HRT	Withdrew	40%	no change	3.0%

Mean difference = +2.6% = δ_0 so n > 94

However, non-attenders = 50% and n must be doubled to 188 in each group